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Postprandial increases in nitrogenous excretion and urea synthesis in the giant mudskipper *Periophthalmodon schlosseri*

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Summary

The objective of this study was to determine the effects of feeding on the excretory nitrogen (N) metabolism of the giant mudskipper, *Periophthalmodon schlosseri*, with special emphasis on the role of urea synthesis in ammonia detoxification. The ammonia and urea excretion rates of *P. schlosseri* increased 1.70- and 1.92-fold, respectively, within the first 3 h after feeding on guppies. Simultaneously, there were significant decreases in ammonia levels in the plasma and the brain, and in urea contents in the muscle and liver, of *P. schlosseri* at 3 h post-feeding. Thus, it can be concluded that *P. schlosseri* was capable of unloading ammonia originally present in some of its tissues in anticipation of ammonia

released from the catabolism of excess amino acids after feeding. Subsequently, there were significant increases in urea content in the muscle, liver and plasma (1.39-, 2.17- and 1.62-fold, respectively) at 6 h post-feeding, and the rate of urea synthesis apparently increased 5.8-fold between 3 h and 6 h. Increased urea synthesis might have occurred in the liver of *P. schlosseri* because the greatest increase in urea content was observed therein. The excess urea accumulated in the body at 6 h was completely excreted between 6 and 12 h, and the percentage of waste-N excreted as urea-N increased significantly to 26% during this period, but never exceeded 50%, the criterion for ureotely, meaning that *P. schlosseri* remained ammonotelic after feeding. By 24 h, 62.7% of the N ingested by *P. schlosseri* was excreted, out of which 22.6% was excreted as urea-N. This is the first report on the involvement of increased urea synthesis and excretion in defense against ammonia toxicity in the giant mudskipper, and our results suggest that an ample supply of energy resources, e.g. after feeding, is a prerequisite for the induction of urea synthesis. Together, increases in nitrogenous excretion and urea synthesis after feeding effectively prevented a postprandial surge of ammonia in the plasma of *P. schlosseri* as reported previously for other fish species. Consequently, contrary to previous reports, there were significant decreases in the ammonia content of the brain of *P. schlosseri* throughout the 24 h period post-feeding, accompanied by a significant decrease in brain glutamine content between 12 h and 24 h.

Key words: amino acids, ammonia, ammonia excretion, feeding, glutamine, mudskipper, nitrogen metabolism, ornithine–urea cycle, *Periophthalmodon schlosseri*, urea.

Introduction

When confronted with aerial exposure, tropical air-breathing fishes would be expected to have difficulties excreting ammonia, resulting in an accumulation of toxic ammonia in the body. In the mid-1960s, there was a concerted effort to extend the known principles of ammonia detoxification by urea synthesis in African lungfishes and amphibians to these air-breathing fishes (Graham, 1997). Malcolm Gordon and coworkers (Gordon et al., 1968, 1969; Gordon, 1970) were the first to investigate if induction of urea synthesis occurred in mudskippers during aerial exposure.

Mudskippers (*Periophthalmus* spp., *Boleophthalmus* spp., *Scartelaos* spp. and *Periopthalmodon* spp.) are euryhaline and amphibious gobioid teleosts (Order: Perciformes, and Family Gobiidae) usually found in mangrove swamps and estuaries. They are highly adaptable to different environmental conditions (Clayton, 1993; Chew et al., 2004). The mud deposited by the river at the estuary forms a suitable habitat for these mudskippers to thrive and build their burrows. During the breeding season, the female lays eggs inside the burrow, and the male stays therein to take care of the developing embryos.

The giant mudskipper *Periophthalmodon schlosseri* can be found on muddy shores in estuaries and in the tidal zone of rivers in Singapore (Ip et al., 1990), Indonesia, New Guinea, India, Peninsular Malaysia, Sarawak and Thailand (Murdy, 1989). It is carnivorous and can grow up to 27 cm in length. *Periophthalmodon schlosseri* is the only species of mudskipper that has not been found outside the tropics. It can survive aerial

exposure much better than other mudskippers (Ip et al., 1993; Kok et al., 1998), in part due to its specialized gill morphology and morphometry (Low et al., 1988, 1990; Wilson et al., 1999, 2000). While other mudskipper species build burrow on soft mud and disappear into the burrow twice a day during high tides, *P. schlosseri* builds its burrow on high ground and usually swims along the water's edge when the tide is high.

Gordon et al. (1969, 1978) reported that when the mudskipper *Periophthalmus sorbinus* was exposed to terrestrial conditions for 12 h, urea production increased more than threefold. However, Gregory (1977) could not detect activity of some ornithine–urea cycle (OUC) enzymes, including carbamoyl phosphate synthetase (CPS), argininosuccinate synthetase and argininosuccinate lyase from the liver of *Periophthalmus expeditionium*, *Periophthalmus gracilis*, and *Scartelaos histophorous*. It was therefore concluded that urea was produced in livers of these mudskippers through uricolysis, involving urate oxidase, allantoinase and allantoicase. The activities of arginase and urate oxidase in their livers are high enough to account for the rate of urea excretion (Gregory, 1977). Working on the mudskippers *Periophthalmus modestus* (as *P. cantonensis*) and *Boleophthalmus pectinitrostris*, Morii (1979) and Morii et al. (1978, 1979) reported that ammonia was not detoxified to urea in these mudskippers during aerial exposure. Iwata et al. (1981) and Iwata (1988) also reported that urea production remained unchanged in *P. modestus* exposed to environmental ammonia or terrestrial conditions. When *P. modestus* was exposed to ^{15}N-labelled ammonia, urea-N was only slightly labelled (Iwata and Deguichi, 1995). Recently, Lim et al. (2001) confirmed that no N-acetylglutamate activated CPS activity could be detected (detection limit=0.001 μmol min^{-1} g^{-1}) from the liver mitochondria of *Boleophthalmus boddaerti*. Taking all these results together, it can be concluded that urea synthesis *de novo* may not occur in *Periophthalmus* spp., *Scartelaos* spp. or *Boleophthalmus* spp.

To date, the only mudskipper that possesses a full complement of hepatic OUC enzymes, in spite of uncertainty on the type of mitochondrial CPS present, is the giant mudskipper *P. schlosseri* (Lim et al., 2001). However, similar to other mudskipper species, detoxification of ammonia to urea does not occur in *P. schlosseri* confronted with adverse environmental conditions such as aerial exposure (Ip et al., 1993; Lim et al., 2001), alkaline environmental pH (Chew et al., 2003) and environmental ammonia (Peng et al., 1998; Randall et al., 1999). Instead, *P. schlosseri* adopts other strategies to defend against ammonia toxicity (Ip et al., 2001a, in press; Randall et al., 2004; Chew et al., 2004). It is capable of actively excreting NH_4^+ against an ammonia concentration (Randall et al., 1999; Ip et al., 2004a) or in a medium with alkaline pH (Chew et al., 2003), manipulating the pH of the external environment (Chew et al., 2003; Ip et al., 2004a), and altering the phospholipid composition of its skin to reduce the influx of NH_3 during environmental ammonia exposure (Ip et al., in press). In addition, it can detoxify ammonia to glutamine when exposed to high concentrations of environmental ammonia (Peng et al., 1998), and reduce ammonia production and undergo partial amino acid catabolism during aerial exposure (Ip et al., 2001b, Lim et al., 2001). With the development of all these mechanisms, it remains an enigma as to why there is still the need to express the OUC in the liver of adult *P. schlosseri*.

Although the expression of OUC is known to occur in fish embryos (Depeche et al., 1979; Wright, 1995; Chadwick and Wright, 1999; Terjesen et al., 2000), the presence of a functional OUC in the liver of adult teleosts is rare, except for the Lake Magadi tilapia *Alcolapia grahami* (Randall et al., 1989), the gulf toadfish *Opsanus beta* (Mommsen and Walsh, 1989; Anderson and Walsh, 1995) and certain catfishes (*Heteropneustes fossilis* and *Clarias batrachus*) from India (Saha and Ratha, 1994; Saha et al., 1997, 1999; for a contrary view on *C. batrachus*, see Ip et al., 2004b; Chew et al., 2004). Being the only mudskipper that is carnivorous (other species are either herbivorous or omnivorous), we suspected that the presence of the OUC in *P. schlosseri* could be related to its high protein diet (mangrove crabs and small fishes), and is involved in the fish's defence against postprandial ammonia toxicity. A postprandial surge in plasma ammonia level is known to occur in several fish species (Kaushik and Teles, 1985; Wicks and Randall, 2002). Therefore, this study was undertaken to determine the effects in *P. schlosseri* of feeding on nitrogen (N) excretion and metabolism, with special emphasis on the role of urea synthesis in ammonia detoxification. The hypothesis tested was that feeding would induce increased urea synthesis and urea excretion in this mudskipper.

Materials and methods

Animals

Periophthalmodon schlosseri Pallas (30–110 g body mass) were captured at Pontian, Malaysia, and transferred to Singapore. They were maintained in plastic aquaria in 50% (15‰ salinity) seawater at 25°C in the laboratory, and the seawater was changed daily. No attempt was made to separate the sexes. The fish were acclimated to laboratory conditions for 1 week. During the adaptation period, *P. schlosseri* were fed small guppies (*Poecilia reticulata*). Food was withdrawn 96 h prior to experiments, which gave sufficient time for the gut to be emptied and a high probability that feeding would then occur. All experiments were performed under a 12 h:12 h dark:light regime.

Feed analysis

The wet mass of guppies was obtained to the nearest mg. Samples of guppies were then freeze-dried and the dry mass recorded. Subsequently, they were analyzed for nitrogen (N) and carbon (C) using a Eurovector EA3011 Elemental Analyzer (Milan, Italy) equipped with the Callidus software. BBOT ($C_{26}H_{26}N_2O_2S$) standard obtained from Eurovector was used as a standard for comparison. In addition some samples were extracted in 70% ethanol for 24 h to remove non-protein

N-compounds, before freeze-drying for nitrogen and carbon analyses. The difference in values between samples with and without ethanol extraction revealed the combined contribution of ammonia, urea, free amino acids (FAAs), purines and pyrimidines to the N and C contents of the guppy.

To determine the content of ammonia, urea, FAAs and protein-bound amino acids (PAAs) in guppies, samples were weighed, ground to a powder in liquid nitrogen, and homogenized using an Ultra-Turrax homogenizer (Janke and Kundel, Staufeni, Staufen, Germany) in 5 volumes (w/v) of 6% trichloroacetic acid (TCA) at 24 000 r.p.m. three times for 20 s each, with a 10 s interval between each homogenization. The homogenate was centrifuged at 10 000 ***g*** at 4°C for 15 min to obtain the supernatant and precipitated proteins.

The pH of the supernatant was adjusted to 5.5–6.0 with 2 mol l^{-1} $KHCO_3$. Ammonia was assayed using the method of Bergmeyer and Beutler (1985). Urea was determined colorimetrically by the method of Jow et al. (1999). For FAA analysis, the supernatant obtained was adjusted to pH 2.2 with 4 mol l^{-1} lithium hydroxide and diluted appropriately with 0.2 mol l^{-1} lithium citrate buffer (pH 2.2). FAAs were analyzed using a Shimadzu LC-6A amino acid analysis system (Kyoto, Japan) with a Shim-pack ISC-07/S1504 Li-type column (Kyoto, Japan).

The precipitated proteins were hydrolyzed with 4 mol l^{-1} methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole (Pierce, Rockford, IL, USA) under vacuum in Pierce hydrolysis tubes at 115°C for 22 h by the method of Simpson et al. (1976). The hydrolysate was centrifuged, adjusted to pH 2.2 with 4 mol l^{-1} lithium hydroxide and diluted appropriately with 0.2 mol l^{-1} lithium citrate buffer (pH 2.2) for analysis by the Shimadzu LC-6A amino acid analysis system.

Despite performing complete FAA and PAA analyses on the guppy samples, only the contents (μmol g^{-1} wet mass) of free and protein-bound arginine, total FAA and total PAA are presented here.

Feeding the animals

Specimens were divided into two groups. The first group of control fish was not fed while the second group of experimental fish was allowed to feed on guppies to 1.5% of their body mass *ad libitum*. The experiment was considered to commence (time = 0 h) when the fish stopped feeding upon satiation. The fed fish and control fish were gently transferred to individual tanks containing 800 ml of 50% seawater. The actual mass of feed consumed by the fish was then calculated by subtracting the mass of any leftover food from the initial mass of food given to the fish.

Collection of water, tissue samples and feed for analyses

Water samples (3 ml) were collected at 3 h intervals during the subsequent 24 h period post-feeding, acidified with 70 μl of 1 mol l^{-1} HCl, and kept at 4°C until analysis. At 0, 3, 6, 12 and 24 h, fish were killed by a strong blow to the head, and the lateral muscle, liver, gut and brain quickly excised. The gut was removed, flushed well with water, and divided into two halves longitudinally. The excised tissues and organs (<1 g) were immediately freeze-clamped in liquid nitrogen using pre-cooled tongs (Faupel et al., 1972). Frozen samples were kept at –80°C until analysis. Blood samples were collected from the severed caudal peduncle into heparinized capillary tubes, and centrifuged at 5000 ***g*** and 4°C for 5 min to obtain the plasma. The plasma was deproteinized by the addition of an equal volume (v/v) of ice-cold 6% TCA and centrifuged at 10 000 ***g*** and 4°C for 15 min. The resulting supernatant was kept at –25°C until analysis.

The frozen samples were weighed, ground to a powder in liquid nitrogen, and homogenized using the Ultra-Turrax homogenizer in 5 volumes (w/v) of ice-cold 6% perchloric acid at 24 000 r.p.m. three times, 20 s each, 10 s interval between each homogenization. The homogenate was centrifuged at 10 000 ***g*** at 4°C for 30 min, and the supernatant obtained was kept at –25°C until analysis.

Determination of ammonia and urea concentrations in water samples

Ammonia in water samples was determined by the method of Anderson and Little (1986), and urea content was analyzed as described by Jow et al. (1999). The rates of ammonia or urea excreted were expressed as μmol N 3 h^{-1} g^{-1} wet mass of the fish.

Determination of ammonia, urea and glutamine in tissues samples

The deproteinized tissue samples were adjusted to pH 6.0–6.5 with 2 mol l^{-1} $KHCO_3$. Ammonia and urea contents were determined using the method of Bergmeyer and Beutler (1985) and Jow et al. (1999), respectively. Urea content in the brain was not determined because of the small size of the brain sample. Glutamine was determined by the method of Mecke (1985). Results were expressed as μmol g^{-1} wet mass tissue or μmol ml^{-1} plasma.

Statistical analyses

Results are presented as means ± standard errors of the mean (S.E.M.). Two-tail Student's *t*-test and one-way analysis of variance followed by Duncan's multiple-range test were used to evaluate differences between means, where applicable. Arcsine transformation was applied to percentage data before statistical analysis. Differences with $P<0.05$ were regarded as statistically significant.

Results

For every 1 g wet mass of guppy ($N=3$), there was 0.25 g of freeze-dried material, of which 8.82±0.17% and 42.3±1.2% of the dry mass were N and C, respectively. After ethanol extraction, the percentages of dry mass represented by N and C were 8.48±0.25 and 40.9±2.1, respectively, indicating that proteins were the major contributor of N. The ammonia and urea contents (μmol g^{-1} wet mass) of guppies were 2.6±0.1

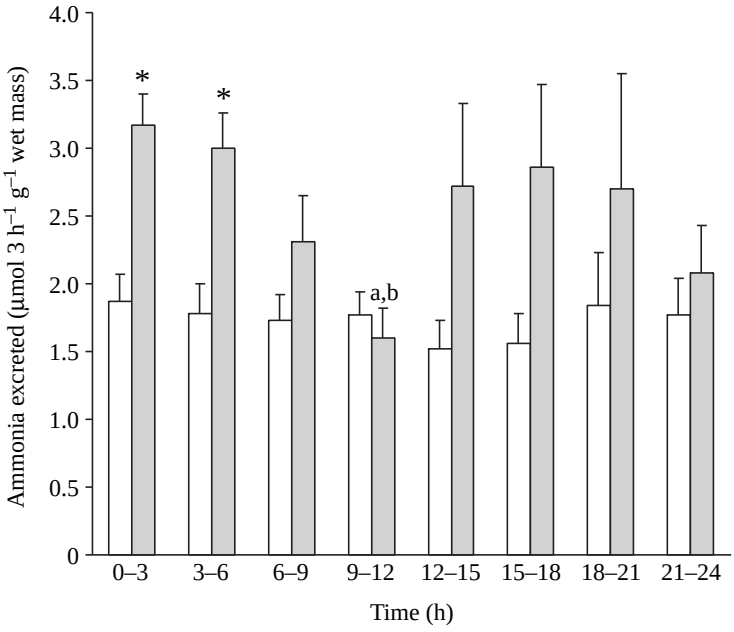

Fig. 1. The amount of ammonia excreted (μmol N 3 h^{-1} g^{-1} wet mass fish) by unfed (control; white squares) and fed (grey squares) *Periophthalmodon schlosseri* at 3 h intervals during the 24 h period post-feeding. Values are means + S.E.M. (*N*=4). *Significantly different from the corresponding control value, $P<0.05$. [a]Significantly different from 0–3 h, $P<0.05$; [b]significantly different from 3–6 h, $P<0.05$.

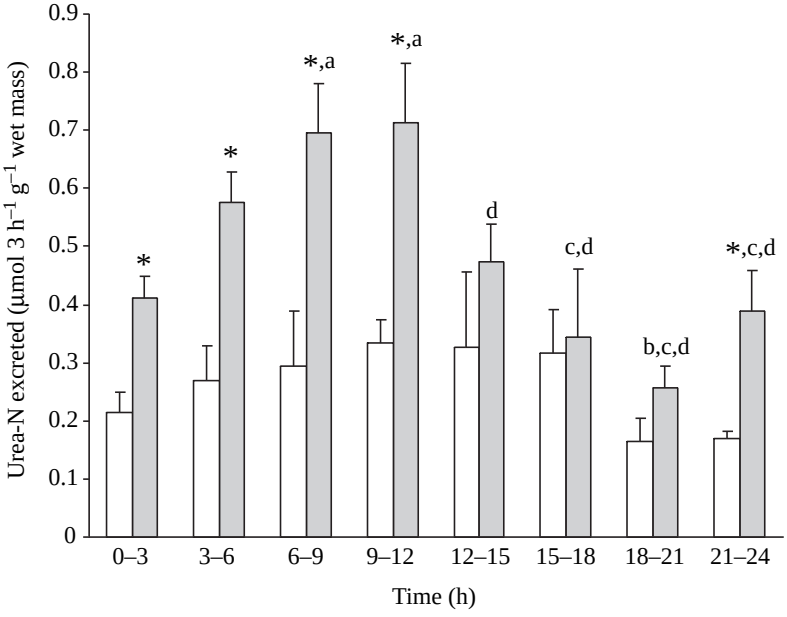

Fig. 2. The amount of urea excreted (μmol N 3 h^{-1} g^{-1} wet mass fish) by unfed (control; white squares) and fed (grey squares) *Periophthalmodon schlosseri* at 3 h intervals during the 24 h period post-feeding. Values are means + S.E.M. (*N*=4). *Significantly different from the corresponding control value, $P<0.05$. [a]Significantly different from 0–3 h, $P<0.05$; [b]significantly different from 3–6 h, $P<0.05$; [c]significantly different from 6–9 h, $P<0.05$; [d]significantly different from 9–12 h, $P<0.05$.

and 1.1±0.1, respectively. The free and protein-bound arginine contents were 0.53±0.19 and 73±2, respectively. The total PAA content (1490±58 μmol g^{-1} wet mass) was 34-fold greater than the total FAA content (44±3 μmol g^{-1} wet mass). The contribution of arginine to the total FAA and total PAA contents were 1.2% and 4.9%, respectively.

The mean body mass of *P. schlosseri* (*N*=17) and the wet mass of the guppies ingested were 95.8±6.9 g and 0.76±0.06 g, respectively. After feeding, a significant increase in the ammonia excretion rate occurred immediately between 0 and 6 h. The ammonia excretion rate of the experimental animals was greatest between 0 and 3 h (3.18 μmol N 3 h^{-1} g^{-1} wet mass fish), and was approximately 1.7-fold greater than the corresponding control value (Fig. 1). Overall, the amount of ammonia excreted within the 24 h period for fed fish (19.5 μmol N 24 h^{-1} g^{-1} wet mass) was significantly greater than the unfed control (13.8 μmol N 24 h^{-1} g^{-1} wet mass).

The rate of urea excretion also increased immediately after feeding, and the increase lasted for 12 h (Fig. 2). The greatest urea excretion rate was observed at 12 h, reaching 0.714 μmol N 3 h^{-1} g^{-1} wet mass, which was 2.14-fold greater than the corresponding control value (Fig. 2). The urea excretion rates between 12 and 21 h were comparable to the corresponding controls, but increased significantly again at 21–24 h post-feeding (Fig. 2). The amount of urea excreted within the 24 h period for fed specimens (3.74 μmol N 24 h^{-1} g^{-1} wet mass) was significantly greater than in the unfed control (2.08 μmol N 24 h^{-1} g^{-1} wet mass). Specifically between 6 and 12 h, the amount of total-N excreted as urea-N increased to 26%, which was approximately 1.60-fold greater than the corresponding control value (Fig. 3).

Ammonia content of the muscle remained relatively unchanged during the 24 h period post-feeding (Fig. 4). In contrast, there was a 2.2-fold increase in ammonia content in the liver at 6 h (Fig. 4), and a slight but significant increase in ammonia content in the gut at 3 h and 6 h (Fig. 4). In the brain, there were significant decreases in the ammonia content throughout the 24 h period post-feeding (Fig. 4). The ammonia concentration in the plasma decreased significantly at 3 h and returned to the normal level thereafter (Fig. 4).

The urea contents of the muscle and liver of *P. schlosseri* at 3 h post-feeding were significantly lower than the corresponding 0 h control value, but by 6 h had increased significantly (Fig. 5). There was also a significant increase in plasma urea

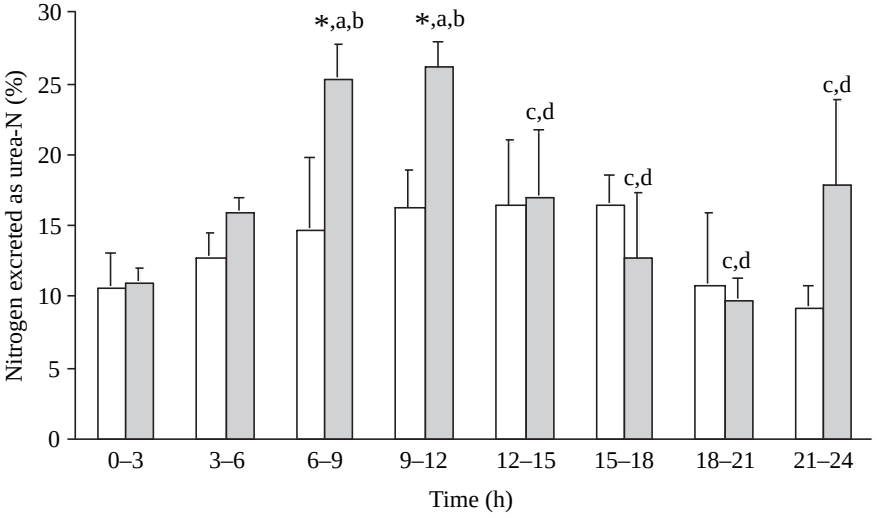

Fig. 3. The percentage of total-N excreted as urea-N by unfed (control; white squares) and fed (grey squares) *Periophthalmodon schlosseri* at 3 h intervals during the 24 h period post-feeding. Values are means + S.E.M. (N=4). *Significantly different from the corresponding control value, P<0.05. [a]Significantly different from 0–3 h, P<0.05; [b]significantly different from 3–6 h, P<0.05; [c]significantly different from 6–9 h, P<0.05; [d]significantly different from 9–12 h, P<0.05.

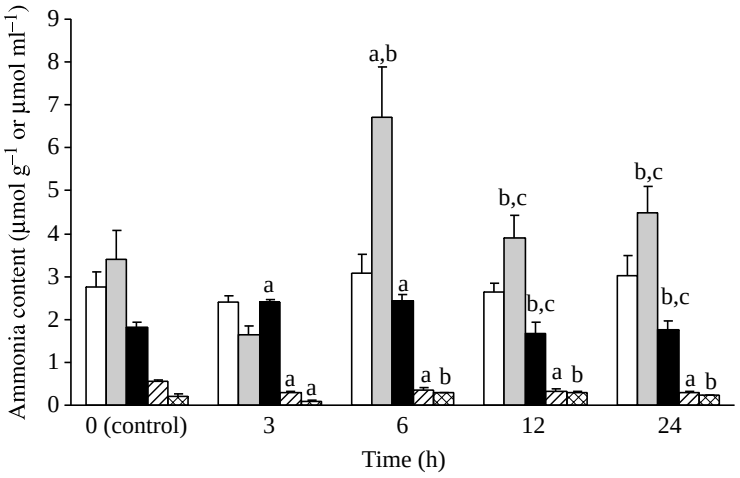

Fig. 4. Ammonia content (µmol g^{-1} wet mass tissue or µmol ml^{-1} plasma) in muscle (white bars), liver (grey bars), gut (black bars), brain (hatched bars) and plasma (cross-hatched bars) of *Periophthalmodon schlosseri* during the 24 h period post-feeding. Values are means + S.E.M. (N= 4), except for brain and plasma (N=3). [a]Significantly different from 0 h control, P<0.05; [b]significantly different from 3 h value, P<0.05; [c]significantly different from 6 h value, P<0.05.

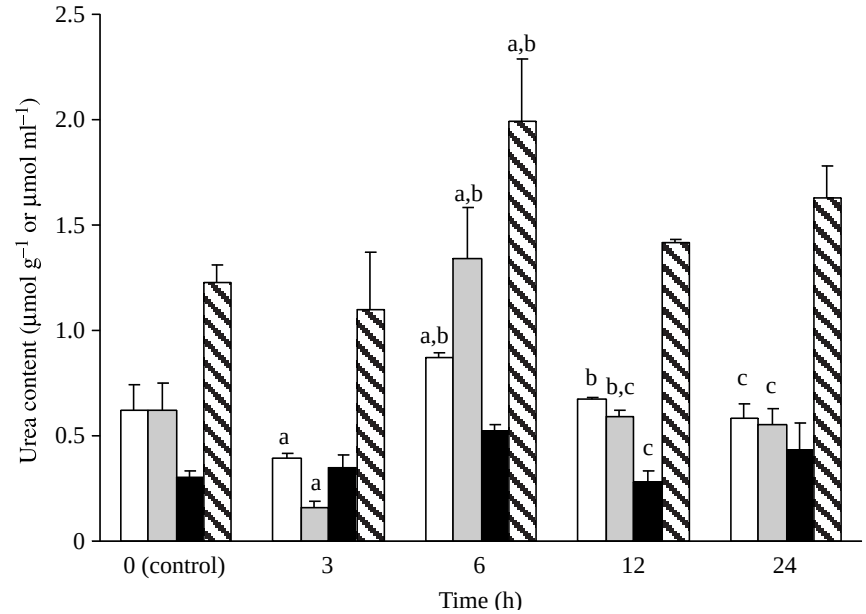

Fig. 5. Urea content (µmol g^{-1} wet mass tissue or µmol ml^{-1} plasma) in muscle (white bars), liver (grey bars), gut (black bars) and plasma (hatched bars) of *Periophthalmodon schlosseri* during the 24 h period post-feeding. Values are means + S.E.M. (N= 3), except for gut (N=4). [a]Significantly different from 0 h control, P<0.05; [b]significantly different from 3 h value, P<0.05; [c]significantly different from 6 h value, P<0.05.

concentration in fed fish at 6 h (Fig. 5). By contrast, the urea content of the gut remained relatively constant throughout the 24 h period post-feeding (Fig. 5).

Feeding had no significant effect on the glutamine content in muscle of *P. schlosseri*, but the glutamine content in the liver significantly decreased at 3 h and 6 h. In addition, brain glutamine content significantly decreased in the fed fish at 12 h and 24 h post-feeding (Fig. 6). By contrast, there was a significant increase in the glutamine content in the gut at 3 h post-feeding (Fig. 6).

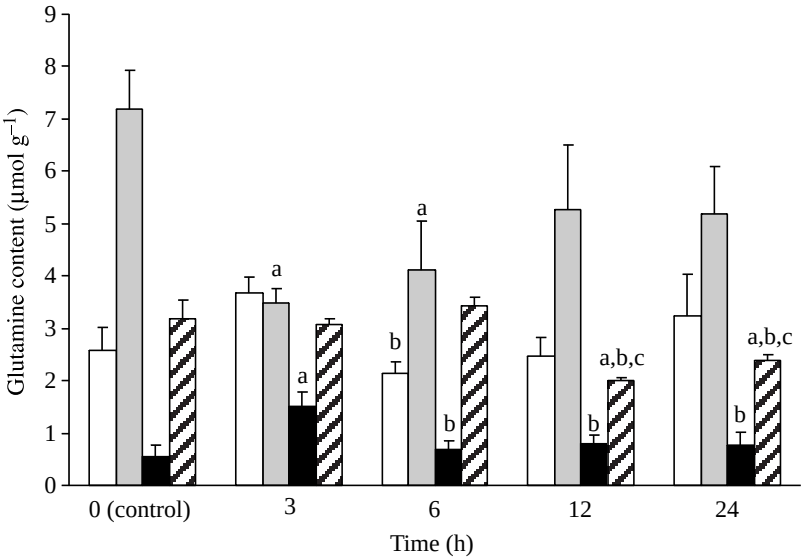

Fig. 6. Glutamine contents (μmol g^{-1} wet mass tissue) in the muscle (white bars), liver (grey bars), gut (black bars) and brain (hatched bars) of the giant mudskipper, *Periophthalmodon schlosseri* during the subsequent 24 h period post-feeding. Values are means + S.E.M. (N=4), except for the brain (N=3). [a]Significantly different from 0 h control, P<0.05; [b]significantly different from 3 h value, P<0.05; [c]significantly different from 6 h value, P<0.05.

Discussion

Feeding induced instantaneous increases in ammonia and urea excretion in P. schlosseri

After consumption of a protein-containing meal, FAAs produced by the actions of proteases in the alimentary tract and peptidases in the intestinal mucosal cells (Mathews, 1975) enter the circulation. The majority of these amino acids, in excess of what is required for protein synthesis, are catabolized in the liver (Campbell, 1991), releasing ammonia and resulting in a momentarily increase in plasma ammonia level in fish (Kaushik and Teles, 1985; Wicks and Randall, 2002), associated with changes in ammonia excretion in fish during the postprandial period; the rate of ammonia excretion usually increases between 2 h and 11 h post-feeding (van Weerd et al., 1995; Dosdat et al., 1996; Gelineau et al., 1998).

In rainbow trout *Onchorhynchus mykiss*, the plasma ammonia concentration increases to 37 μg ml^{-1} or 2.07 μmol ml^{-1} 8 h after feeding (Wicks and Randall, 2002). By contrast, the rates of ammonia and urea excretion in the giant mudskipper increased by 1.70- and 1.92-fold, respectively, within the first 3 h post-feeding. In fact, the greatest rate of ammonia excretion was observed at 0–3 h and returned back to normal at 6–9 h. Since there were significant decreases in ammonia levels in plasma and brain of *P. schlosseri* at 3 h post-feeding, these results indicate that *P. schlosseri* had unloaded the ammonia originally present in some of its tissues immediately after feeding (probably initiated by the feeding action involved), in anticipation of ammonia being released by catabolism of excess amino acids. In addition, there was a 1.4-fold increase in ammonia excretion over the 24 h period, with the majority excreted between 12 h and 21 h. This unique pattern of ammonia excretion is part of a novel phenomenon: unlike in other fish species (Kaushik and Teles, 1985; Wicks and Randall, 2002), there was no postprandial surge in ammonia concentration in the plasma of *P. schlosseri* during the 24 h post-feeding period. Other factors contributing to this novel phenomenon were increased synthesis and excretion of urea (see below) in *P. schlosseri* after feeding.

A significant increase in the rate of urea excretion in *P. schlosseri* also occurred immediately after feeding, and lasted for 12 h. At 3 h post-feeding, the urea contents in the muscle and liver decreased by 36.6% and 74.7%, respectively. Taken together, these results indicate that feeding induced an instantaneous increase in the rate of nitrogenous excretion (ammonia + urea) in *P. schlosseri*.

A hypothetical *P. schlosseri* weighing 70 g would have consumed 0.53 g of guppies or 0.84 mmol of N. By 24 h post-feeding, a total of 0.52 mmol (calculated from Figs 1 and 2) or 62.7% of the ingested N would have been excreted by this 70 g fish. Out of this 0.52 mmol N excreted, 22.6% (0.116 mmol) would be urea-N, whereas in control (unfed) fish only 13.1% of the waste-N would be excreted as urea-N. The percentage of urea-N actually excreted increased to 26% between 6 and 12 h in the fed fish, and since there were significant increases in urea content in the muscle and liver at 6 h after the initial ‘unloading’ of urea at 3 h, it can be deduced that an increase in urea production had occurred in *P. schlosseri* between 3 h and 6 h post-feeding.

Increased urea synthesis in P. schlosseri *after feeding*

The urea excretion rate increased approximately twofold in *P. schlosseri* at 6–12 h post-feeding. In addition, the urea contents of muscle, liver and plasma increased 1.39-, 2.17- and 1.62- fold, respectively, at 6 h, before returning back to control values at 12 h. These results suggest that urea production increased in *P. schlosseri* after feeding. Furthermore, it is apparent that the increased rate of urea

production must have been greater than that of urea excretion, in order for urea to be accumulated in the tissues of the fed fish.

To maintain the concentration of urea in the body of the control animal at a steady state, the rate of urea excretion must balance the rate of urea production. This implies that the hourly rate of urea production in *P. schlosseri* at 0 h was 0.036 μmol h^{-1} g^{-1} [0.216 urea-N/(3 × 2 N); from Fig. 2], or 2.52 μmol h^{-1} for a hypothetical 70 g fish.

Upon feeding, a 70 g fish would have excreted 20.1 μmol urea between 3 h and 6 h (0.57 urea-N g^{-1} × 70 g/2 N; from Fig. 2). Based on values of 42 g muscle, 2 g liver and 2 ml plasma in a 70 g fish (Lim et al., 2001), the excess amount of urea accumulated between 3 h and 6 h can be calculated as (0.47 μmol g^{-1} × 42 g) + (1.18 μmol g^{-1} × 2 g) + (0.90 μmol ml^{-1} × 2 ml) or 23.9 μmol 70 g^{-1} fish (from Fig. 5). Thus, the amount of urea produced by a 70 g fish during this 3 h period post-feeding is equal to the sum of the amount excreted and the amount accumulated in the body, which is 20.1 + 23.9 or 44 μmol. The hourly rate of urea production in a 70 g specimen between 3 h and 6 h post-feeding is therefore 44 μmol 3 h^{-1} or 14.7 μmol h^{-1}, which means that the urea production rate increased 5.8-fold (=14.7 μmol h^{-1} *vs.* 2.52 μmol h^{-1}), with the production of 36.5 μmol urea in excess, within this 3 h period.

Urea can be produced *via* uricolysis, argininolysis or the OUC (Campbell, 1973), but only production *via* the OUC can be regarded as a synthetic process. Uric acid can be produced *via* purine catabolism; however, purine, together with other ethanol-extractable nitrogenous compounds, only had a minor contribution to the total-N in guppies (0.32% of dry mass, or 30.2 μmol N per 0.53 g guppies, of which at least 23.3 μmol N was contributed by FAA). Therefore, degradation of purine from ingested guppies apparently could not account for the amount of 58.1 μmol urea produced during the 24 h period post-feeding (see above). The amounts of arginine present as FAA and PAA in 0.53 g of guppies consumed by a 70 g *P. schlosseri* were 0.28 and 38.7 μmol, respectively. Since one mole of arginine gives rise to one mole of urea, at most 39 μmol of urea could be produced through argininolysis, based on the highly unlikely assumption that both free and protein-bound arginine were selectively and completely catabolized in preference to other amino acids. Even then, this (39 μmol) could account for only 67% of the 58.1 μmol urea produced during the 24 h period. Therefore, it can be concluded that a major portion of the urea produced by *P. schlosseri* after feeding was actually synthesized *de novo via* the OUC. Urea synthesis is likely to have occurred in the liver because the greatest increase in urea content was observed therein. Since there is 2 g of liver in a 70 g fish, the rate of urea synthesis in the liver between 3 h and 6 h post-feeding is equal to 14.7 μmol h^{-1}/(60 min × 2 g) or 0.123 μmol min^{-1} g^{-1}, which is close to the highest CPS activities reported for the liver of *P. schlosseri* (0.117 μmol min^{-1} g^{-1}; Lim et al., 2001).

Attempts had been made previously to elucidate the mechanisms adopted by *P. schlosseri* to ameliorate ammonia toxicity during exposure to terrestrial conditions (Ip et al., 1993, 2001b; Lim et al., 2001), alkaline environmental pH (Chew et al., 2003) or environmental ammonia (Peng et al., 1998; Randall et al., 1999; Ip et al., 2004a). Despite possessing all the enzymes for urea synthesis *de novo* in its liver, *P. schlosseri* is apparently incapable of detoxifying ammonia to urea when exposed to these experimental conditions. Thus, our results represent the first report on the involvement of increased urea synthesis and excretion in the defense against postprandial ammonia toxicity in the giant mudskipper. Since all previous studies (Peng et al., 1998; Randall et al., 1999; Chew et al., 2003; Ip et al., 1993, 2001b; Lim et al., 2001) on *P. schlosseri* were performed using fasted specimens, our results suggest that, perhaps, an ample supply of energy resources, e.g. after feeding, is a prerequisite for the induction of urea synthesis.

P. schlosseri can survive aerial exposure much better than other species of mudskippers (Ip et al., 1993; Kok et al., 1998) partly because of its specialized gill morphology and morphometry (Low et al., 1988, 1990; Wilson et al., 1999, 2000); but fusions of secondary lamellae would impose inefficiency in the branchial excretion of ammonia in water. Therefore, in addition to an increase in the rate of ammonia excretion after feeding, increased urea synthesis is essential in preventing a postprandial surge of ammonia. However, *P. schlosseri* remained ammonotelic throughout the 24 h period post-feeding. With the excess urea accumulated in the body at 6 h being completely excreted between 6 and 12 h, the percentage of waste-N excreted as urea-N increased significantly to 26%, but never exceeded 50%, the criterion for ureotely. In this respect, *P. schlosseri* is different from the ammonotelic, but ureogenic, slender African lungfish *Protopterus dolloi*, which becomes ureotelic after feeding (Lim et al., in press).

Ammonia and glutamine contents in brain of P. schlosseri *decreased significantly after feeding*

The mechanisms involved in defense against postprandial ammonia toxicity in *P. schlosseri* were so effective that the ammonia level in the brain decreased throughout the 24 h period. Consequently, *P. schlosseri* exhibited a phenomenon different from other fishes with respect to the response of brain glutamine content to feeding. It is well known that fish brains are protected from ammonia toxicity by glutamine synthetase (Mommsen and Walsh, 1991), and that ammonia levels in the fish brain increases significantly after feeding, leading to increased glutamine synthesis and its accumulation therein (Wicks and Randall, 2002; Lim et al., in press). By contrast, feeding led to a significant decrease in the brain glutamine level of *P. schlosseri* between 12 h and 24 h. This observation is consistent with the fact that there was an absence of any postprandial ammonia surge in the plasma throughout the 24 h period post-feeding.

Conclusion

The rates of ammonia and urea excretion increased significantly in *P. schlosseri* after feeding. It would appear that *P. schlosseri* was capable of unloading ammonia and urea from its body (over a 0–3 h period post-feeding) in anticipation of an increase in ammonia production from catabolism of excess amino acid. In addition, the rate of urea synthesis increased 5.8-fold between 3 h and 6 h. These adaptations effectively prevented a postprandial surge of ammonia in *P. schlosseri*, as has been reported previously for other fish species. Consequently, there were significant decreases in the ammonia content in the brain of *P. schlosseri* throughout the 24 h period post-feeding. In addition, unlike in other fish species, the brain glutamine content decreased significantly after feeding (12–24 h). Similar to *P. schlosseri*, certain adult teleosts such as the largemouth bass *Micropterus salmoides* and the plainfin midshipman *Porichthys notatus* are known to be ureogenic (for a review, see Anderson, 2001), although it has been suggested that the OUC and CPS in these ammonotelic fishes may not have a significant physiologically function (Anderson, 2001). Perhaps future work should aim to elucidate if urea synthesis *de novo* plays an important role in defense against postprandial ammonia toxicity in these ureogenic fishes as reported herein for the giant mudskipper *P. schlosseri*.

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