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The swamp eel *Monopterus albus* reduces endogenous ammonia production and detoxifies ammonia to glutamine during 144 h of aerial exposure

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Summary

The swamp eel *Monopterus albus* inhabits muddy ponds, swamps, canals and rice fields, where it can burrow within the moist earth during the dry summer season, thus surviving for long periods without water. This study aimed to elucidate the strategies adopted by *M. albus* to defend against endogenous ammonia toxicity when kept out of water for 144 h (6 days). Like any other fish, *M. albus* has difficulties in excreting ammonia during aerial exposure. In fact, the rates of ammonia and urea excretions decreased significantly in specimens throughout the 144 h of aerial exposure. At 144 h, the ammonia and urea excretion rates decreased to 20% and 25%, respectively, of the corresponding control values. Consequently, ammonia accumulated to high levels in the tissues and plasma of the experimental specimens. Apparently, *M. albus* has developed relatively higher ammonia tolerance at the cellular and subcellular levels compared with many other teleost fish. Since the urea concentration in the tissues of specimens exposed to air remained low, urea synthesis was apparently not adopted as a strategy to detoxify endogenous ammonia during 144 h of aerial exposure. Instead, ammonia produced through amino acid catabolism was detoxified to glutamine, leading to the accumulation of glutamine in the body during the first 72 h of aerial exposure. Complimenting the increased glutamine formation was a significant increase in glutamine synthetase activity in the liver of specimens exposed to air for 144 h. Formation of glutamine is energetically expensive. It is probably because *M. albus* remained relatively inactive on land that the reduction in energy demand for locomotory activity facilitated its exploitation of glutamine formation to detoxify endogenous ammonia. There was a slight decrease in the glutamine level in the body of the experimental animals between 72 h and 144 h of aerial exposure, which indicates that glutamine might not be the end product of nitrogen metabolism. In addition, these results suggest that suppression of endogenous ammonia production, possibly through reductions in proteolysis and amino acid catabolism, acts as the major strategy to avoid ammonia intoxication in specimens exposed to air for ≥72 h. It is concluded that glutamine formation and reduction in ammonia production together served as effective strategies to avoid the excessive accumulation of ammonia in the body of *M. albus* during 144 h of aerial exposure. However, these strategies might not be adequate to sustain the survival of *M. albus* in the mud for longer periods during drought because ammonia and glutamine concentrations had already built up to high levels in the body of specimens exposed to air for 144 h.

Key words: ammonia, amino acid, *Monopterus albus*, glutamate, glutamine, glutamine synthetase, swamp eel, urea.

Introduction

Ammonia is mainly produced in fish through catabolism of amino acids, and ammonia production occurs mainly in the liver of fish (Pequin and Serfaty, 1963), although other tissues are also capable of doing so (Walton and Cowey, 1977). Ammoniagenesis is achieved mainly by the transamination of amino acids followed by the deamination of glutamate and/or adenylates in fish muscle during severe exercise (Driedzic and Hochachka, 1976). Most aquatic animals keep body ammonia levels low by simply excreting the excess ammonia that has been produced through digestion and metabolism.

In this report, NH_3 represents un-ionized molecular ammonia, NH_4^+ represents ammonium ions, and ammonia refers to both NH_3 and NH_4^+. Ammonia is usually excreted as NH_3 across the body surface, usually the gills, of fish into the surrounding water (Wilkie, 1997). Under acidic environmental conditions, NH_3 diffusing across the gills is converted to NH_4^+ and trapped in the water. Thus, acidic conditions in the environment augment ammonia excretion. The diffusion of ammonia into the environment is inhibited if the pH of the environment is high, and ammonia subsequently accumulates

in the body. Decomposition of organic matter or the use of fertilizers will increase ammonia levels in the water and this will result in elevated ammonia levels in the fish. Finally, excretion is also reduced if the fish moves out of water (reviewed by Ip et al., 2001a).

The swamp eel *Monopterus albus* (Zuiew 1793) is a bony fish (family Synbranchidae; order Synbranchiformes; class Actinopterygii). It is not really an eel because it does not belong to the family Anguillidae of the order Anguilliformes. *M. albus* can be found in the tropics (34° N to 6° S) from India to southern China, Malaysia and Indonesia. It has an anguilliform body reaching a maximal length of 100 cm at maturity, with no scale and no pectoral and pelvic fins, and the dorsal, caudal and anal fins are confluent and reduced to a skin fold. *M. albus* lives in muddy ponds, swamps, canals and rice fields (Rainboth, 1996), where it burrows in moist earth in dry season, surviving for long periods without water during summer (Shih, 1940; Davidson, 1975). During prolonged drought, it burrows deep into the mud to remain in contact with the water table (Liem, 1987). While *M. albus* can tolerate the seasonal draining of rice patties in the mud, it can survive only several days in the market without water (Wu and Kung, 1940). At present, the reason behind this discrepancy in the capability of *M. albus* to survive in mud and in air is not clear. Preliminary observations made in our laboratory confirmed that the mortality of specimens exposed to air rose from 0% after 6 days to 30% after 8 days. Therefore, this study was undertaken to examine the strategies adopted by *M. albus* to defend against ammonia toxicity during 144 h (6 days) of aerial exposure.

Like many other tropical air-breathing fish, when *M. albus* moves on or hides in the mud in the dry seasons it encounters a lack of water. This would lead to difficulties in excreting ammonia through its gills and cutaneous surfaces. Hence, one of the objectives of this study was to examine if aerial exposure would cause the accumulation of ammonia in the body and if *M. albus* could tolerate high levels of ammonia at the tissue and cellular levels.

Ammonia is toxic and affects various cellular processes (Ip et al., 2001a). Therefore, many tropical species have evolved mechanisms to deal with the increased body ammonia loads resulting from reduction in ammonia excretion associated with aerial exposure. These mechanisms include: (1) reducing ammonia production, as in the mudskippers *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*, the four-eyed sleeper *Bostrichyths sinensis*, the weather loach *Misgurnus anguillicaudatus* and the mangrove killifish *Rivulus marmoratus* (Ip et al., 2001a,b,c; Lim et al., 2001; Chew et al., 2001; Frick and Wright, 2002a); (2) undergoing partial amino acid catabolism leading to the accumulation of alanine, as in the giant mudskipper *P. schlosseri* and the snakehead *Channa asiatica*; (3) detoxifying ammonia to urea, as in the African lungfishes *Protopterus aethiopicus* (Janssens and Cohen, 1968) and, possibly, the Indian catfishes *Heteropneustes fossilis* and *Clarias batrachus* (Saha and Ratha, 1998; Anderson, 2001; but see review by Chew et al., in press, for a different view); (4) detoxifying ammonia to glutamine, as in the marble goby *Oxyeleotris marmoratus* and the four-eyed sleeper (Jow et al., 1999; Ip et al., 2001a,b); (5) actively excreting NH_4^+ into water trapped between secondary lamellae of the gills, as in the giant mudskipper (Randall et al., 1999; Chew et al., 2003a); and (6) excreting NH_3 into air, as in the blenny *Alticus kirki*, the weather loach and the mangrove killifish (Rozemeijer and Plaut, 1993; Tsui et al., 2002; Frick and Wright, 2002b). The responses of tropical fish to aerial exposure are many and varied, determined by the behaviour of the fish and the nature of the environment in which it lives. Thus, the additional aims of this study were to examine whether *M. albus* would (1) volatilize NH_3, (2) detoxify ammonia to urea when exposed to terrestrial conditions, (3) be capable of suppressing endogenous ammonia production by reductions in proteolysis and amino acid catabolism, (4) be capable of undergoing partial amino acid catabolism leading to the formation of alanine without releasing ammonia, and (5) be capable of detoxifying ammonia to glutamine while it is out of water. It was hypothesized that *M. albus* had adopted one or more of these strategies to survive 144 h of aerial exposure.

Materials and methods

Collection and maintenance of specimens

Monopterus albus (Zuiew 1793; 200–400 g body mass) were purchased locally from a fish farm in Singapore. They were maintained in plastic aquaria in freshwater (1‰ salinity) at 25°C in the laboratory and fed liver guppy *ad libitum*. No attempt was made to separate the sexes. The eels were acclimated to laboratory conditions for at least one week before experimentation. Food was withdrawn 48 h prior to experiments, which gave sufficient time for the gut to be emptied of all food and waste products.

Experiment 1: does M. albus *volatilize NH_3 during aerial exposure?*

The set-up used by Tsui et al. (2002) for the weather loach was adopted for this experiment, except that a sealed plastic container with a total volume of 1.5 litres was used to house *M. albus*. Specimens (N=8) were kept individually in plastic containers with 1 litre of freshwater (pH 7.0) at 25°C. After 24 h, the water in the container and the acid in the two NH_3 traps were analyzed for ammonia concentration. The containers were then rinsed. The same eels were immediately exposed to air for 24 h in the same container but with only 50 ml of freshwater (pH. 7.0). At the end of the second 24 h period, the water in the container and the acid in the NH_3 traps were collected for ammonia assay. Ammonia concentration was determined colorimetrically according to the method of Anderson and Little (1986).

Experiment 2: effects of aerial exposure on the rates of ammonia and urea excretion in M. albus

For the control (N=4), *M. albus* were exposed to 20 volumes (w/v) of freshwater (pH. 7.0) in plastic containers (50 cm×30 cm×20 cm, length × width × height). Water

samples (3 ml) were collected at 6 h and 24 h, acidified with 0.07 ml of 1 mol l^{-1} HCl to prevent the loss of NH_3 and stored at 4°C. Ammonia and urea assays were performed within 48 h of sample collection. Preliminary results indicated that the rates of ammonia and urea excretion were linear within the 24 h period. Water was changed daily after the collection of the 24 h sample. The experiment lasted 144 h.

For experimental specimens (N=4) exposed to air, *M. albus* were kept in similar plastic containers but with only 200 ml of freshwater (pH 7.0). Water was sampled at 24 h, after which the container was rinsed and new freshwater (200 ml) was added. After 144 h of aerial exposure, the fish was immersed in 20 volumes (w/v) of freshwater to study the rates of ammonia and urea excretion upon recovery from aerial exposure. Analyses of ammonia and urea concentration were performed within 48 h using the methods described above. Ammonia concentration was determined colorimetrically according to the method of Anderson and Little (1986). Urea was assayed according to the method of Jow et al. (1999).

Experiment 3: effects of aerial exposure on ammonia, urea and free amino acid (FAAs) concentration and enzyme activity in M. albus

Tissue preparation

A group of specimens (N=6) were killed at the start of the experiment to act as 0 h controls. Another group (N=2–5) was kept in 20 volumes (w/v) of freshwater (pH 7.0) for 6 days and served as the 144 h controls. Other specimens (N=20) were exposed to air in plastic containers (50 cm×30 cm×20 cm, length × width × height) with only a thin film of freshwater (200 ml; pH 7.0). Specimens were killed after 24 h, 72 h or 144 h of air exposure. The water was changed daily in all cases.

For the collection of plasma, the caudal peduncle of the fish was severed, and blood exuding from the caudal artery was collected in sodium heparin-coated Eppendorf tubes. The plasma obtained after centrifugation at 5000 ***g*** at 4°C for 5 min was deproteinized by adding 2 volumes (v/v) of ice-cold 6% trichloroacetic acid (TCA) and centrifuged at 10 000 ***g*** at 4°C for 10 min. The resulting supernatant was kept at –80°C for analysis of ammonia, urea and FAAs. The brain, liver, lateral muscle and gut (flushed thoroughly with saline) were excised and immediately freeze-clamped with tongs pre-cooled in liquid nitrogen. Samples were stored at –80°C until analysis.

Analysis of ammonia, urea and FAA concentration

For ammonia, urea and FAA analysis, the frozen liver, muscle and gut tissue samples were weighed and pulverised to a powder at –80°C. Five volumes of ice-cold 6% TCA were added and the mixture was homogenized three times for 20 s each (with 10 s intervals) with an Ultra-Turrax homogenizer (Janke & Kunkel GmBH and Co., Staufen, Germany) at 24 000 revs min^{-1}. Frozen brain samples were weighed and then hand-homogenized in 10 volumes (w/v) of 6% TCA using a glass-pestle homogenizer (Wheaton Science Products, Millville, NJ, USA). The samples were then centrifuged at 10 000 ***g*** at 4°C for 10 min, and the supernatants were stored at –80°C for subsequent analysis. Ammonia and urea assays were performed within 2 weeks, and FAA analysis was completed within one month.

For ammonia analysis, the pH of the deproteinized sample was adjusted to 6.0–6.5 with 2 mol l^{-1} $KHCO_3$. Ammonia concentration was determined as described by Kun and Kearney (1974). Freshly prepared NH_4Cl solution was used as the standard for comparison. Urea concentration in a 0.2 ml deproteinized sample was analyzed colorimetrically according to the method of Jow et al. (1999). Urea (Sigma, St Louis, MO, USA) was used as a standard. Results are expressed as μmol g^{-1} wet mass tissue or μmol ml^{-1} plasma.

For FAA analysis, deproteinized muscle, liver, brain, gut and plasma samples were thawed and diluted with an equal volume of 2 mol l^{-1} lithium citrate buffer and adjusted to pH 2.2 with 4 mol l^{-1} LiOH. These samples were then analyzed for FAA concentration using an LC-6A Amino Acid Analysis System with a Shim-pack ISC-07/S1504 Li-type column (Shimadzu, Nakagyo-ku, Kyoto, Japan). The concentrations of FAAs are expressed as μmol g^{-1} wet mass for brain, liver, muscle and gut samples and as μmol ml^{-1} for plasma samples; the total FAA (TFAA) concentration is expressed as the sum of the FAAs.

Analysis of enzyme activity

For enzyme assays, the frozen muscle, liver and gut samples were weighed and homogenized three times in 5 volumes (w/v) of ice-cold extraction buffer, containing 50 mmol l^{-1} imidazole-HCl (pH 7.0), 50 mmol l^{-1} NaF and 3 mmol l^{-1} EGTA, at 24 000 revs min^{-1} for 20 s with 10 s intervals. The homogenate was sonicated three times for 20 s with 10 s intervals and then centrifuged at 10 000 ***g*** at 4°C for 20 min. The supernatant was then passed through a 10 ml Econo-Pac 10DG desalting column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) equilibrated with 50 mmol l^{-1} imidazole-HCl (pH 7.0). The resulting eluent was used for enzymatic analysis. The protein concentration of the extract was measured before and after filtration to calculate the dilution factor involved. The frozen brain sample was weighed and then homogenized in 5 volumes (w/v) of ice-cold extraction buffer using a Wheaton glass-pestle homogenizer. The homogenate was centrifuged at 10 000 ***g*** for 15 min at 4°C. The supernatant was dialyzed against a buffer containing 50 mmol l^{-1} imidazole-HCl (pH 7.0) using Microdialyzer System 100 (Pierce, Rockford, IL, USA). The dialysate was used for enzyme analyses. Enzyme analyses were recorded with a Shimadzu UV-1601 UV–VIS recording spectrophotometer at 25°C. All chemicals and coupling enzymes were obtained from Sigma.

Glutamine synthetase (GS; EC 6.3.1.2) activity was determined colorimetrically according to the method of Shankar and Anderson (1985). GS activity was expressed as μmol γ-glutamyl hydroxamate formed min^{-1} g^{-1} wet mass tissue. Freshly prepared glutamic acid monohydroxamate solution was used as a standard for comparison.

The activities of alanine aminotransferase (ALT; EC 2.6.1.2), which catalyses alanine degradation, and aspartate

aminotransferase (AST; EC 2.6.1.1), which catalyses aspartate degradation, were determined according to Peng et al. (1994). Glutamate dehydrogenase (GDH; EC 1.4.1.3) activity, which catalyses amination, was assayed according to Ip et al. (1993). ALT, AST and GDH activities were monitored at 340 nm. Enzyme activities were expressed in μmol NADH utilized min^{-1} g^{-1} wet mass.

Statistical analyses

Results were presented as means ± S.E.M. Student's *t*-test and one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls multiple range test were used to compare differences between means where applicable. Differences with $P<0.05$ were regarded as statistically significant.

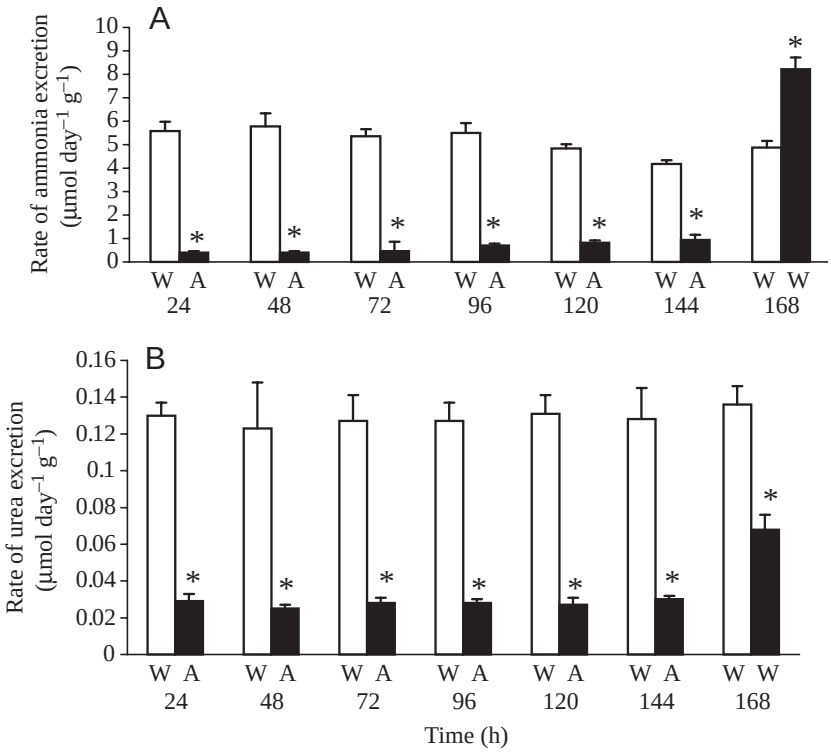

Fig. 1. Effects of aerial exposure on the rates of (A) ammonia and (B) urea excretion (μmol day^{-1} g^{-1}) of *Monopterus albus*. Values are means + S.E.M. (N=4). W, in water; A, in air; *****significantly different from the values of the corresponding control in water at $P<0.05$.

Results

The rate of ammonia excretion by *M. albus* during aerial exposure was significantly lower than when the fish was submerged. It was ten times less than the corresponding submerged control at 24 h and five times less than the control value at 144 h (Fig. 1A). Upon recovery in water, the ammonia excretion rate rose to 1.5 times that of its corresponding control value (Fig. 1A). Aerial exposure also significantly lowered the rate of urea excretion to 25% that of the control value (Fig. 1B). Only 0.003±0.002% and 0.100±0.070% of total ammonia was liberated as ammonia gas by *M. albus* in water or in air, respectively (Table 1).

Ammonia levels in the muscle (Fig. 2A), liver (Fig. 2B), brain (Fig. 2C), gut (Fig. 2D) and plasma (Fig. 2E) of *M. albus* increased significantly during aerial exposure. After 72 h of aerial exposure, ammonia concentration in the liver, brain and plasma were 3-fold, 3.5-fold and 5-fold, respectively, those of the control values. In the muscle and gut, the ammonia concentration reached the highest level of 6.9 μmol g^{-1} and 4.5 μmol g^{-1}, respectively, after 144 h of aerial exposure. These were approximately 3.5 times that of the control values (Fig. 2A,D).

Urea concentration did not increase significantly in the muscle, liver and plasma of eels after 144·h of aerial exposure compared with the 144 h water controls (Fig. 3A,B,E). The urea concentration in the brain of the specimens exposed to air for 144 h was 1.5-fold that of the 144 h control (Fig. 3C). In the gut, the urea concentration rose after 24 h of aerial exposure but that of specimens exposed to air for 144 h was comparable with that of the 144 h control in water (Fig. 3D).

In the muscle, glutamine concentration peaked after 72 h of aerial exposure and was 4.5-fold higher than that of the 0 h control value by 144 h (Table 2). Significant increases were also observed in the concentrations of alanine, histidine, isoleucine, leucine, methionine, serine, taurine, threonine, tyrosine and valine after 72 h of air exposure compared to 0 h

Table 1. *Concentrations of ammonia dissolved in the external environment and ammonia gas evolved and trapped in acid, and the percentage of total ammonia excreted as ammonia gas when* Monopterus albus *was kept in freshwater or exposed to air for 24 h*

Conditions	Ammonia dissolved in FW (μmol g^{-1} fish)	Ammonia gas trapped in acid (μmol g^{-1} fish)	Ammonia/total ammonia (%)
Water	105.02±8.46	0.00299±0.00193	0.003±0.002
Air	8.33±0.30[a]	0.00805±0.00515	0.100±0.070

Results represent means ± S.E.M. (N=8)
[a]Significantly different from the submerged control value.

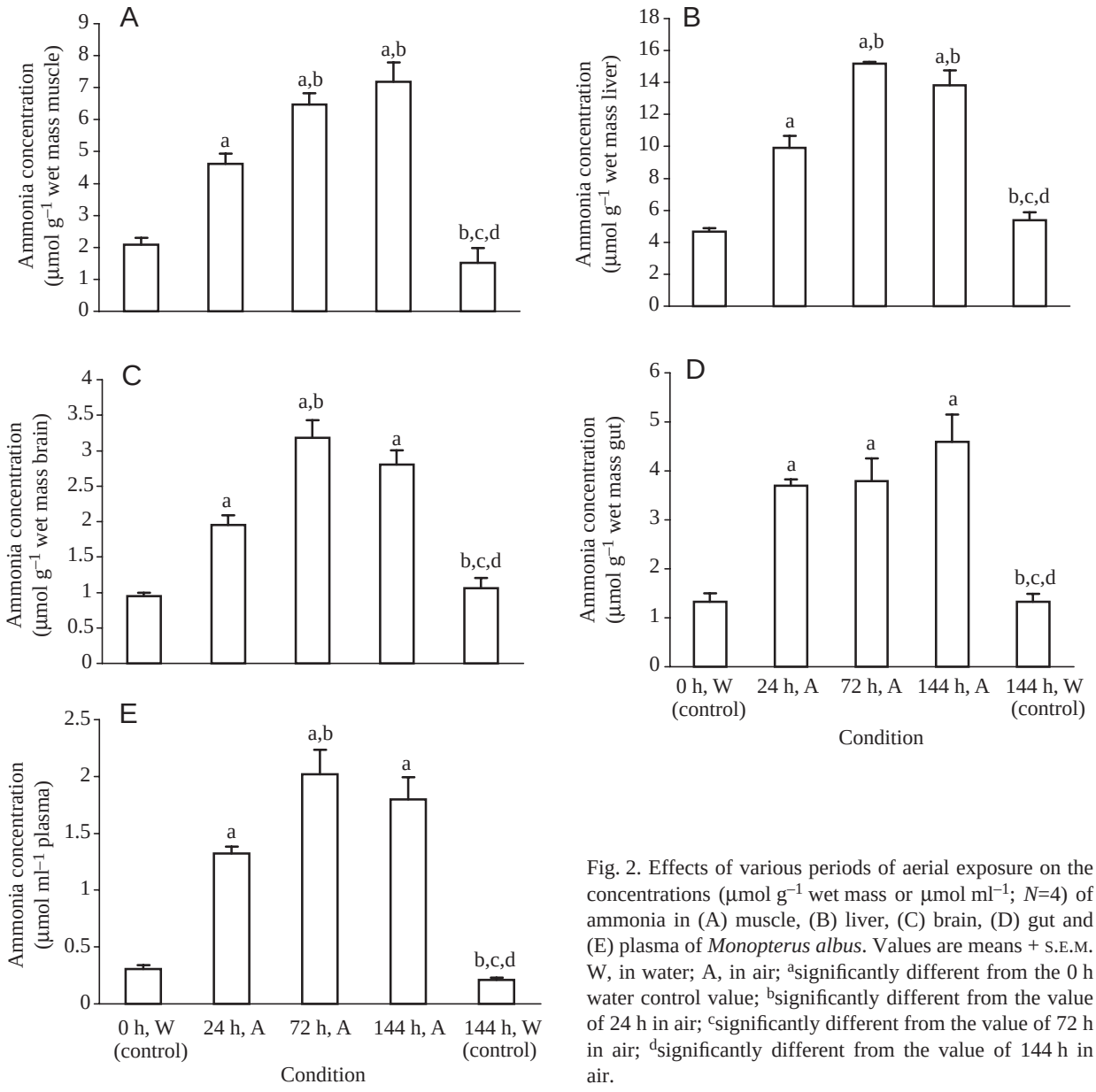

Fig. 2. Effects of various periods of aerial exposure on the concentrations (μmol g^{-1} wet mass or μmol ml^{-1}; *N*=4) of ammonia in (A) muscle, (B) liver, (C) brain, (D) gut and (E) plasma of *Monopterus albus*. Values are means + S.E.M. W, in water; A, in air; [a]significantly different from the 0 h water control value; [b]significantly different from the value of 24 h in air; [c]significantly different from the value of 72 h in air; [d]significantly different from the value of 144 h in air.

control (Table 2). However, by 144 h, these values had either returned to control levels (histidine, isoleucine, leucine, taurine, threonine, tyrosine and valine) or fallen to levels lower than those of the 144 h control (alanine, methionine and serine) (Table 2). The total free amino acid (TFAA) content in specimens exposed to air for 144 h was comparable with that of the 144 h control in water (Table 2).

In the liver, the glutamine concentration compared with the 0 h water control increased 36- and >29-fold by 72 h and 144 h, respectively (Table 3). At 144 h, the levels of alanine, histidine and tyrosine were markedly lower than the corresponding control values (Table 3). However, the TFAA content of specimens exposed to 144 h terrestrial conditions was comparable with that of the 144 h control.

In the brain, the glutamine level increased significantly to 4-fold the 0 h control values after 72 h of aerial exposure (Table 4). The concentrations of leucine, lysine, proline, serine and tyrosine were also affected after exposure to air for 144 h compared with the 144 h water control (Table 4). Similar large increases in glutamine levels were also observed in the gut and plasma of air-exposed specimens (Tables 5, 6).

After 144 h of aerial exposure, GS and GDH (which catalyse amination) activities were halved in the muscle of *M. albus*, reaching 1.09±0.17 μmol min^{-1} g^{-1} and 0.06±0.01 μmol min^{-1} g^{-1}, respectively (Table 7). In the liver, there were marked increases in the activities of GS and AST after 144 h of aerial exposure, approaching 1.4- and 1.3-fold the corresponding control value (Table 7). In the brain, significant decreases in ALT and AST activities were observed (Table 7). In the gut, ALT activity rose significantly from

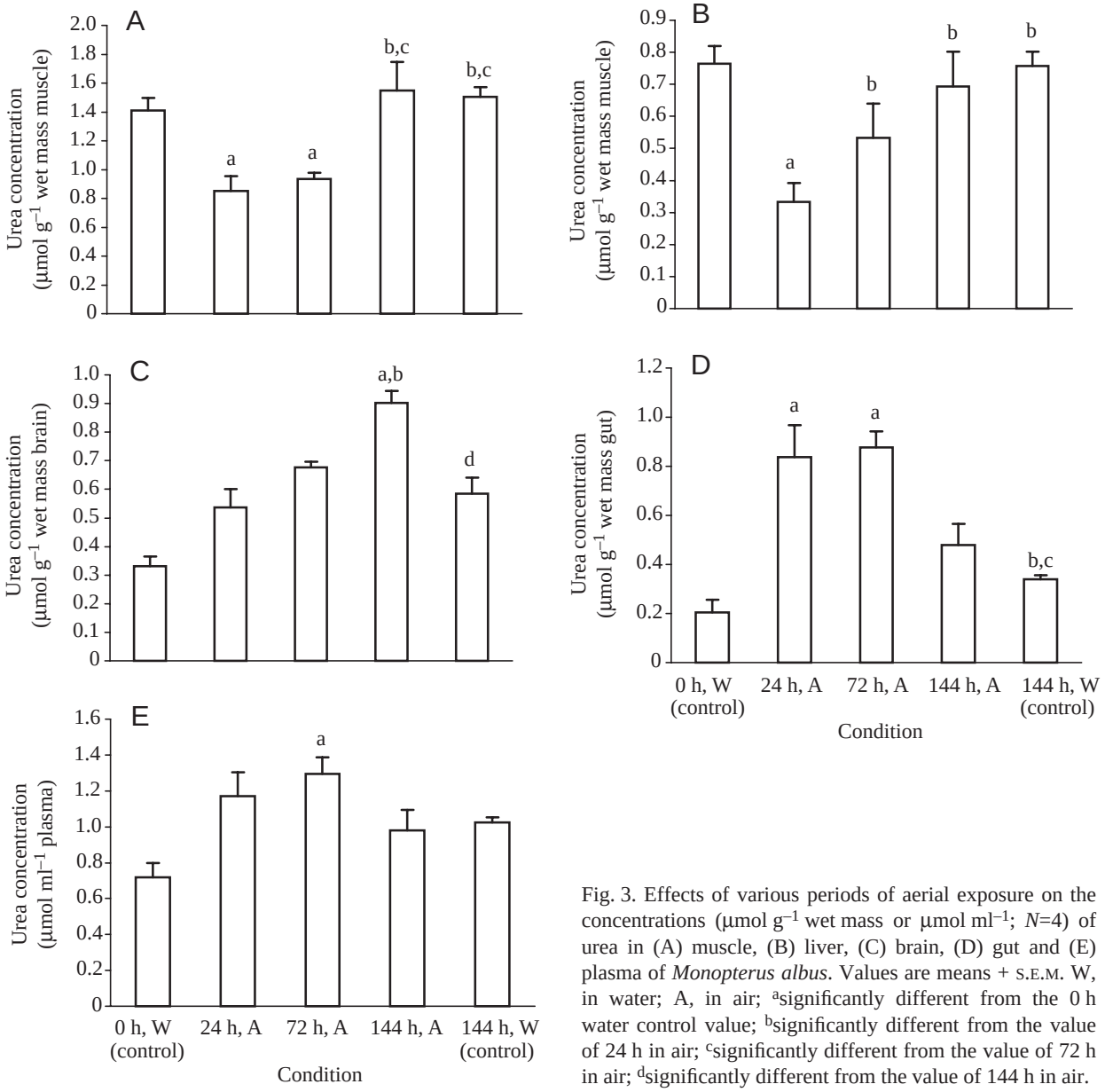

Fig. 3. Effects of various periods of aerial exposure on the concentrations (μmol g^{-1} wet mass or μmol ml^{-1}; *N*=4) of urea in (A) muscle, (B) liver, (C) brain, (D) gut and (E) plasma of *Monopterus albus*. Values are means + S.E.M. W, in water; A, in air; [a]significantly different from the 0 h water control value; [b]significantly different from the value of 24 h in air; [c]significantly different from the value of 72 h in air; [d]significantly different from the value of 144 h in air.

1.00±0.05 μmol min^{-1} g^{-1} to 1.47±0.05 μmol min^{-1} g^{-1} after 144 h of aerial exposure (Table 7).

Discussion

Ammonia excretion is reduced on land, and M. albus *is incapable of volatilizing NH_3*

M. albus was ammonotelic in water, with ammonia-N excretion accounting for >97% of the total-N (ammonia-N + urea-N) excreted (Fig. 1; Table 8). However, branchial ammonia excretion would not be possible when there is no water to irrigate the gills during aerial exposure (Wilkie, 1997; Sayer and Davenport, 1987). Hence, the rate of ammonia excretion in *M. albus* decreased significantly during aerial exposure (Fig. 1A). Consequently, this led to an accumulation of ammonia in the body of the specimens kept away from water. This in turn might lead to a slight increase in ammonia excretion rate on land with time.

In a number of terrestrial ammonotelic invertebrates, NH_3 volatilization contributes significantly to total ammonia elimination and also water conservation (e.g. Wright et al., 1993; Greenaway and Nakamura, 1991). Amongst vertebrates, however, terrestrial ammonotely is uncommon and examples of significant NH_3 volatilization are rare. In teleost fish, the first report of ammonia volatilization was in the temperate intertidal blenny (*Blennius pholis*) but it only accounted for 8% of the total ammonia excreted during emersion (Davenport and Sayer, 1986). However, there are a few studies on ammonotelic tropical fish capable of enduring emersion and volatilizing significant amounts of ammonia (Rozemeijer and Plaut, 1993;

Table 2. *Effects of various periods of aerial exposure on the concentrations of various free amino acids (FAAs) and total FAA (TFAA) in the muscle of* Monopterus albus

	FAA concentration (μmol g^{-1} wet mass)				
FAA	Water 0 h	Air 24 h	Air 72 h	Air 144 h	Water 144 h
Alanine	0.513±0.042	0.605±0.081	1.258±0.260[a,b]	0.428±0.023[c]	1.269±0.093[a,b,d]
Asparagine	0.425±0.047	0.245±0.015[a]	0.561±0.072[b]	0.560±0.071[b]	0.457±0.027[b]
Aspartate	0.100±0.005	0.076±0.009	0.126±0.018	0.096±0.007	0.669±0.091[a,b,c,d]
Arginine	0.569±0.091	0.392±0.034[a]	0.676±0.063[b]	0.748±0.094[b]	0.457±0.019
Glutamine	1.609±0.314	3.641±0.615	10.112±0.943[a,b]	7.616±0.365[a,b,c]	1.486±0.269[c,d]
Glutamate	0.468±0.082	0.548±0.065	0.294±0.020	0.546±0.121	1.243±0.208[a,b,c,d]
Glycine	6.187±0.320	4.783±0.506	6.302±0.371	5.025±0.153	5.902±0.401
Histidine	0.160±0.036	0.252±0.009	0.606±0.072[a,b]	0.546±0.079[a,b]	0.469±0.015[a]
Isoleucine	0.116±0.007	0.286±0.011[a]	0.357±0.025[a]	0.232±0.005[a,c]	0.266±0.005[a]
Leucine	0.235±0.025	0.492±0.020[a]	0.603±0.039[a]	0.400±0.007[c]	0.430±0.011[a]
Lysine	1.202±0.089	1.517±0.161	1.797±0.068	1.574±0.105	2.095±0.147[a]
Methionine	0.049±0.004	0.032±0.002[a]	0.064±0.006[a,b]	0.019±0.003[a,c]	0.042±0.002[c,d]
Phenylalanine	0.524±0.025	0.063±0.004[a]	0.061±0.010[a]	0.060±0.005[a]	0.082±0.006[a]
Proline	0.103±0.009	0.134±0.018	0.152±0.023	0.113±0.004	0.172±0.008[a]
Serine	0.156±0.019	0.264±0.033[a]	0.538±0.034[a,b]	0.400±0.049[a,b,c]	0.517±0.026[a,b,d]
Taurine	5.001±0.510	6.642±0.992	10.757±0.956[a,b]	6.502±0.226[c]	5.448±0.233[c]
Threonine	0.158±0.032	0.302±0.038	0.544±0.071[a,b]	0.562±0.071[a,b]	0.561±0.021[a,b]
Tyrosine	0.080±0.003	0.044±0.003	0.180±0.032[a,b]	0.041±0.006[c]	0.061±0.001[c]
Valine	0.197±0.011	0.407±0.009[a]	0.459±0.026[a]	0.340±0.004[a]	0.397±0.018[a]
TFAA	17.852±4.241	20.579±4.700	35.448±8.300[a,b]	25.616±5.713[c]	22.021±4.184[c]

Results represent means ± S.E.M. (N=3).

[a]Significantly different from the value of 0 h, water control; [b]significantly different from the value of 24 h, air; [c]significantly different from the value of 72 h, air; [d]significantly different from the value of 144 h, air.

Frick and Wright, 2002b; Tsui et al., 2002). High temperatures and humidity, characteristic of the tropical climates and the experimental conditions in this study, are factors that increase the likelihood of the ammonia excreted into the film of water covering the body surface being volatilized in significant quantities. The most important factor is the effect of temperature on evaporation rate. Higher temperatures will also decrease the ammonia equilibrium constant (pK_{amm}), resulting in a higher fraction of NH_3 at a given pH. Although *M. albus* is a tropical fish living in environments of high temperature (25–34°C), the low percentage of total ammonia excreted as ammonia gas showed that it was unable to eliminate significant amounts of ammonia in gaseous form during aerial exposure (Table 1).

Ammonia is not detoxified to urea during aerial exposure

There was an apparent increase in the percentage of total-N excreted as urea when *M. albus* was exposed to terrestrial conditions (Fig. 1; Table 8). However, this was mainly due to the drastic decrease of the ammonia excretion rate during aerial exposure. In fact, aerial exposure also affected the rate of urea excretion, which decreased to 25% of the control value (Fig. 1B). However, in contrast to ammonia, the changes in urea concentrations in various tissues and organs examined were relatively minor (Fig. 3A–E). After 24 h of aerial exposure, the urea concentration in the muscle decreased significantly, producing a deficit of –134 μmol urea-N per 200 g fish (Table 8). It is uncertain of the fate of this amount of urea because the rate of urea excretion actually decreased and urease is known to be absent from tissues of vertebrates. Nonetheless, these results suggest that *M. albus* does not adopt urea synthesis as a strategy to defend against endogenous ammonia toxicity during 144 h of aerial exposure.

Contrary to the belief that there is a tendency towards predominance of ureotelism in amphibious species (Mommsen and Walsh, 1989, 1992; Wright, 1995; Saha and Ratha, 1998; Wright and Land, 1998), only a few teleosts are ureotelic, and the majority of adult tropical fish studied so far do not adopt ureogenesis as a major strategy to detoxify endogenously produced ammonia during aerial exposure. These include the mudskippers *P. schlosseri* and *B. boddaerti* (Lim et al., 2001), the marble goby *O. marmoratus* (Jow et al., 1999), the four-eyed sleeper *B. sinensis* (Ip et al., 2001c), the weather loach *M. anguillicaudatus* (Chew et al., 2001), the snakehead *C. asiatica* (Chew et al., 2003b) and the mangrove killifish *R. marmoratus* (Frick and Wright, 2002b) exposed to terrestrial conditions for various periods. Through the present study, one more species can be added to this list – *M. albus*. The synthesis of urea *de novo* in fish is highly energy dependent. A total of 5 moles of ATP are hydrolyzed to ADP for each mole of urea synthesized, corresponding to 2.5 moles of ATP used for each

Table 3. *Effects of various periods of aerial exposure on the concentrations of various free amino acids (FAAs) and total FAA (TFAA) in the liver of* Monopterus albus

	FAA concentration (μmol g^{-1} wet mass)				
FAA	Water 0 h	Air 24 h	Air 72 h	Air 144 h	Water 144 h
Alanine	0.350±0.026	0.094±0.020[a]	0.077±0.006[a]	0.066±0.008[a]	0.182±0.033[a,b,c,d]
Asparagine	0.353±0.038	0.483±0.031	0.780±0.106[a,b]	0.611±0.078[a]	0.627±0.065[a]
Aspartate	0.237±0.031	0.316±0.039	0.292±0.007	0.304±0.026	0.217±0.035
Arginine	0.129±0.009	0.083±0.005[a]	0.090±0.011[a]	0.129±0.008[b,c]	0.078±0.005[a,d]
Glutamine	0.318±0.047	6.066±0.532[a]	11.520±1.265[a,b]	9.397±1.542[a,b]	2.443±0.590[b,c,d]
Glutamate	1.596±0.095	1.680±0.115	1.467±0.039	2.021±0.255	2.283±0.143
Glycine	1.505±0.163	1.151±0.092	0.356±0.040[a,b]	1.108±0.100[c]	1.574±0.294[c]
Histidine	0.167±0.009	0.091±0.012[a]	0.103±0.004[a]	0.101±0.005[a]	0.147±0.016[b,c,d]
Isoleucine	0.037±0.006	0.159±0.028[a]	0.220±0.016[a]	0.158±0.017[a]	0.173±0.012[a]
Leucine	0.306±0.034	0.366±0.041	0.423±0.029	0.306±0.034	0.343±0.006
Lysine	0.214±0.026	0.111±0.006[a]	0.132±0.013[a]	0.185±0.012[b]	0.203±0.008[b,c]
Methionine	0.005±0.001	0.005±0.001	0.010±0.001[a,b]	0.016±0.002[a,b,c]	0.017±0.001[a,b,c]
Phenylalanine	0.049±0.003	0.044±0.001	0.039±0.002	0.031±0.002[a]	0.031±0.004[a]
Proline	0.060±0.011	0.027±0.002[a]	0.027±0.002[a]	0.056±0.007[b,c]	0.065±0.006[b,c]
Serine	0.113±0.007	0.088±0.004	0.086±0.003	0.126±0.020	0.153±0.037
Taurine	2.553±0.150	2.240±0.189	2.306±0.080	2.294±0.107	2.035±0.074
Threonine	0.207±0.012	0.171±0.046	0.185±0.038	0.306±0.026[b]	0.435±0.039[a,b,c]
Tyrosine	0.192±0.016	0.176±0.012	0.211±0.013	0.073±0.006[a,b,c]	0.143±0.005[a,c,d]
Valine	0.204±0.012	0.345±0.040	0.323±0.050	0.309±0.022	0.418±0.030[a]
TFAA	8.595±1.678	13.696±3.567	18.648±6.549[a]	17.595±5.477[a]	11.565±2.084

Results represent means ± S.E.M. (N=3).

[a]Significantly different from the value of 0 h, water control; [b]significantly different from the value of 24 h, air; [c]significantly different from the value of 72 h, air; [d]significantly different from the value of 144 h, air.

mole of nitrogen assimilated. This may be the major reason why urea synthesis *via* the ornithine urea cycle is rare in adult fish (Ip et al., 2001a).

Endogenous ammonia is detoxified to glutamine during aerial exposure

Ammonia toxicity can be avoided by converting ammonia to glutamine. Glutamine is produced from glutamate and NH_4^+, the reaction catalyzed by GS in the muscle and/or liver. Glutamate may in turn be produced from α-ketoglutarate (α-KG) and NH_4^+, catalysed by GDH, or from α-KG and other amino acids, catalysed by various transaminases. In other words, formation of one glutamine molecule allows the uptake of two ammonia molecules (Campbell, 1973). One mole of ATP is required for the production of every amide group of glutamine *via* GS. If the reaction begins with ammonia and α-KG, every mole of ammonia detoxified would result in the hydrolysis of 2 moles of ATP-equivalent (Ip et al., 2001a). Hence, glutamine formation would be more effective than ureogenesis (2.5 moles of ATP for every mole of nitrogen detoxified) with respect to energy expenditure. More importantly, glutamine is stored within the body after being synthesized, and can be used for other anabolic processes (e.g. purine, pyrimidine and mucopolysaccharides) when the environmental conditions become more favourable. Urea, being a small and uncharged molecule permeable to biomembranes, can be excreted easily upon its synthesis, and ureotely actually represents a loss of both nitrogen and carbon to the environment.

Indeed, *M. albus* appears to have adopted glutamine formation as a major strategy to defend against ammonia toxicity during 144 h of aerial exposure. The glutamine concentrations in the muscle of *M. albus* increased by 6-fold (to 10.11 μmol g^{-1}) and 4.5-fold (to 7.62 μmol g^{-1}) after 72 h and 144 h exposure to terrestrial conditions, respectively (Table 2). In the liver, the increase in glutamine level (39-fold and 31-fold after 72 h and 144 h, respectively) was even more drastic (Table 3).

Sleepers (*O. marmoratus* and *B. sinensis*) belonging to the family Eleotridae can detoxify endogenous ammonia to glutamine in non-cerebral tissues during aerial exposure (Jow et al., 1999; Ip et al., 2001b). Since sleepers remain quiescent on land, the reduction in energy demand for muscular activity may provide them with the opportunity to exploit glutamine formation as a means to detoxify ammonia. Results obtained in the present study proved that glutamine synthesis and accumulation as a strategy to defend against endogenous ammonia exposure is not exclusive to the sleepers. *M. albus*, being an eel, can move on land more effectively than the sleepers. However, it usually remains motionless if undisturbed, especially if it has access to a muddy substratum, in which it will bury itself. Presumably,

Table 4. *Effects of various periods of aerial exposure on the concentrations of various free amino acids (FAAs) and total FAA (TFAA) in the brain of* Monopterus albus

	FAA concentration (μmol g^{-1} wet mass)				
FAA	Water 0 h	Air 24 h	Air 72 h	Air 144 h	Water 144 h
Alanine	0.171±0.031	0.299±0.007[a]	0.290±0.016[a]	0.219±0.009	0.197±0.011[b]
Asparagine	n.d.	n.d.	n.d.	n.d.	n.d.
Aspartate	0.769±0.024	0.715±0.014	0.827±0.037	0.792±0.081	0.659±0.033
Arginine	0.092±0.024	0.079±0.003	0.081±0.007	0.066±0.005	0.107±0.005
Glutamine	1.567±0.242	5.277±0.320[a]	6.821±0.557[a,b]	6.967±0.183[a,b]	1.837±0.048[b,c,d]
Glutamate	3.944±0.253	5.186±0.206[a]	6.044±0.257[a,b]	5.326±0.236[a,c]	5.076±0.264[a,c]
Glycine	0.801±0.027	0.961±0.013	0.819±0.012	0.844±0.035	0.897±0.040
Histidine	0.285±0.040	0.156±0.002[a]	0.194±0.026[a]	0.167±0.004[a]	0.149±0.022[a]
Isoleucine	0.021±0.003	0.091±0.002[a]	0.099±0.008[a]	0.070±0.003[a]	0.047±0.003[b,c]
Leucine	0.060±0.005	0.212±0.009[a]	0.230±0.016[a]	0.173±0.009[a,c]	0.114±0.006[a,b,c,d]
Lysine	0.205±0.020	0.087±0.014[a]	0.102±0.012[a]	0.070±0.010[a]	0.190±0.019[b,c,d]
Methionine	0.010±0.001	0.008±0.001	0.014±0.001	0.017±0.001	0.009±0.001
Phenylalanine	0.042±0.003	0.047±0.007	0.043±0.006	0.046±0.008	0.047±0.004
Proline	0.013±0.003	0.033±0.003	0.085±0.007[a,b]	0.055±0.007[a,c]	0.024±0.002[c,d]
Serine	0.072±0.006	0.120±0.008[a]	0.115±0.006[a]	0.088±0.009[b]	0.121±0.009[a,d]
Taurine	5.276±0.368	7.694±0.571[a]	8.193±0.225[a]	6.782±0.399[a,c]	7.131±0.179[a]
Threonine	0.113±0.016	0.134±0.019	0.152±0.009	0.127±0.012	0.095±0.012
Tyrosine	0.066±0.005	0.070±0.005	0.048±0.001[a,b]	0.069±0.006[c]	0.045±0.002[a,b,d]
Valine	0.039±0.004	0.136±0.007[a]	0.135±0.020[a]	0.106±0.003[a]	0.079±0.005
TFAA	13.545±3.597	21.304±5.607	24.291±6.424[a]	21.982±5.810	16.822±4.758

Results represent means ± S.E.M. (N=3).

[a]Significantly different from the value of 0 h, water control; [b]significantly different from the value of 24 h, air; [c]significantly different from the value of 72 h, air; [d]significantly different from the value of 144 h, air; n.d., not detectable.

this behaviour of *M. albus* facilitates its adoption of glutamine synthesis as a major strategy to handle ammonia toxicity.

The concentrations of glutamine in the tissues and organs of specimens exposed to terrestrial conditions for 144 h were generally lower than those of specimens exposed for 72 h. Assuming that endogenous ammonia production was reduced through suppression of proteolysis and amino acid catabolism (see below), glutamine that had been synthesized and accumulated should at least remain at the same level. This indicates that glutamine might not be an end product for accumulation. Rather, in *M. albus*, it could be mobilized to other compounds yet to be identified for storage under long-term exposure to land. It is essential to elucidate this possibility in future studies and to differentiate the metabolic fates of glutamine during long-term aerial exposure and during subsequent recovery in water.

The brain, liver and gut of M. albus *have a high capacity for GS, which is enhanced by aerial exposure*

The brain of *M. albus* has a very high level of GS activity (Table 7), probably the highest known in fish. In the brain, the ALT and AST activities decreased (Table 7), probably to avoid consuming glutamate in order to save it for the synthesis of glutamine. Elevated plasma ammonia concentration is associated with an increase in glutamine concentration in the brain of *M. albus* and other vertebrates (Ip et al., 2001a; Brusilow, 2002). It has also been suggested, however, that high glutamine levels can contribute to toxicity (Brusilow, 2002). It is proposed that increased glutamine production and accumulation cause increased astrocyte cell volume, leading to cellular dysfunction, brain edema and death. L-Methionine *S*-sulfoximine inhibits glutamine synthetase, reduces edema, attenuates ammonia-induced increases in brain extracellular K^+ and ameliorates ammonia toxicity (Brusilow, 2002). Thus, glutamine formation may either exacerbate or ameliorate ammonia toxicity, depending on the site of formation and the species in question. At present, it is unclear how *M. albus* solves the problems associated with increased glutamine formation when exposed to terrestrial conditions.

Besides the brain, GS activity was also present in the liver, gut and muscle of *M. albus* (Table 7), with decreasing maximal activity (close to V_{max}) in that order. There was a significant increase in the activity of GS in the liver after specimens were exposed to terrestrial conditions for 144 h. Hence, it would appear that the liver is the major site of endogenous ammonia detoxification in *M albus*. This contrasts with exposure to environmental ammonia, during which other tissues and organs are involved (Y. K. Ip, S. L. A. Tay, K. H. Lee and S. F. Chew, manuscript submitted).

Table 5. *Effects of various periods of aerial exposure on the concentrations of various free amino acids (FAAs) and total FAA (TFAA) in the gut of* Monopterus albus

	FAA concentration (μmol g^{-1} wet mass)				
FAA	Water 0 h	Air 24 h	Air 72 h	Air 144 h	Water 144 h
Alanine	0.120±0.002	0.184±0.010[a]	0.182±0.007[a]	0.137±0.015[b,c]	0.145±0.003
Asparagine	0.061±0.011	0.071±0.001	0.096±0.009[a]	0.098±0.006[a]	0.056±0.007[c,d]
Aspartate	0.309±0.068	0.549±0.027	0.600±0.056	0.554±0.084	0.458±0.074
Arginine	0.066±0.021	0.094±0.004	0.086±0.006	0.075±0.006	0.081±0.005
Glutamine	0.724±0.265	0.793±0.042	1.983±0.504[a,b]	1.567±0.204[a]	0.666±0.094[c,d]
Glutamate	0.765±0.102	1.508±0.080[a]	1.259±0.041[a,b]	1.569±0.044[a,c]	1.051±0.042[a,b,d]
Glycine	0.211±0.010	0.262±0.017	0.245±0.023	0.259±0.011	0.268±0.012
Histidine	0.056±0.007	0.046±0.003	0.058±0.005	0.060±0.003	0.054±0.003
Isoleucine	n.d.	n.d.	n.d.	n.d.	n.d.
Leucine	0.090±0.009	0.234±0.026[a]	0.270±0.015[a]	0.218±0.007[a]	0.201±0.018[a]
Lysine	0.066±0.002	0.091±0.001	0.076±0.008	0.070±0.002	0.139±0.021[a,c,d]
Methionine	0.012±0.001	0.010±0.001	0.014±0.003	0.017±0.003[b]	0.012±0.002
Phenylalanine	0.030±0.002	0.050±0.004[a]	0.056±0.004[a]	0.028±0.004[b,c]	0.049±0.004[a,d]
Proline	0.045±0.003	0.063±0.007	0.096±0.011[a,b]	0.058±0.007[c]	0.077±0.005[a]
Serine	0.061±0.010	0.078±0.007	0.090±0.004	0.072±0.008	0.069±0.003
Taurine	4.340±0.192	4.223±0.131	5.049±0.163	4.780±0.315	4.412±0.358
Threonine	0.096±0.005	0.085±0.004	0.124±0.005[b]	0.114±0.004	0.112±0.008
Tyrosine	0.229±0.017	0.459±0.042[a]	0.514±0.028[a]	0.426±0.013[a]	0.395±0.030[a]
Valine	0.131±0.017	0.283±0.027[a]	0.344±0.043[a]	0.251±0.013[a]	0.240±0.021[a]
TFAA	7.411±2.441	9.082±2.419	11.143±2.972	10.354±2.803	8.484±2.480

Results represent means ± S.E.M. (N=3).

[a]Significantly different from the value of 0 h, water control; [b]significantly different from the value of 24 h, air; [c]significantly different from the value of 72 h, air; [d]significantly different from the value of 144 h, air; n.d., not detectable.

M. albus *does not undergo partial amino acid catabolism when exposed to land*

Certain amino acids (e.g. arginine, glutamine, histidine and proline) can be converted to glutamate. Glutamate can undergo deamination catalyzed by GDH, producing NH_4^+ and α-KG (Campbell, 1991). The latter is then fed into the Krebs cycle. Glutamate can also undergo transamination with pyruvate, catalyzed by ALT, producing α-KG and alanine without the release of ammonia (Ip et al., 2001a,b; Chew et al., 2003b). If there were a continuous supply of pyruvate, transamination leading to the formation of alanine would facilitate the oxidation of carbon chains of some amino acids without polluting the internal environment with ammonia. Available information indicates that it is a major strategy adopted mainly by fish that are relatively active on land but not by those that have to deal with ammonia loading situations. In the case of *M. albus* exposed to terrestrial conditions, no accumulation of alanine in the muscle and other tissues was observed (Tables 2–6). Also, there was no change in the activity of ALT in the muscle and liver (Table 7). Hence, it can be concluded that alanine formation was not adopted as a strategy to slow down the release of ammonia under such conditions. In the gut, there was an increase in ALT activity upon aerial exposure, the meaning of which is not clear at this moment (Table 7). Whether *M. albus* would adopt partial amino acid catabolism to facilitate the utilization of amino acid as an energy source during locomotion on land awaits future studies. If it does not adopt such a strategy, then it is probably related to its possible exposure to environmental ammonia in its natural habitat.

M. albus *reduces the rates of proteolysis and amino acid catabolism, and hence the rate of ammonia production, during aerial exposure*

In order to slow down the build up of ammonia internally, fish can augment excretion by decreasing the rate of ammonia production through amino acid catabolism. The steady-state concentrations of FAAs in tissues depend on the rates of degradation and production (through proteolysis or digestion), alteration of which would lead to changes in concentrations of various amino acids. Being able to alter these rates is a valuable strategy to a fish that has to endure short periods of water shortage, because it would slow down the build up of endogenous ammonia.

From the results obtained, a balance sheet on the reduction in nitrogenous excretion and the increase in nitrogenous accumulation (as ammonia, urea and glutamine) was constructed for a 200 g specimen of *M. albus* exposed to various periods (24 h, 72 h and 144 h) of aerial exposure (Table 8). After the first 24 h of aerial exposure, the deficit involved was –1073 + 742 = –331 μmol N. After 72 h of aerial exposure, the discrepancy between the reduction in nitrogenous excretion (3204 μmol N) and the retention of

Table 6. *Effects of various periods of aerial exposure on the concentrations of various free amino acids (FAAs) and total FAA (TFAA) in the plasma of* Monopterus albus

	FAA concentration (μmol g^{-1} wet mass)				
FAA	Water 0 h	Air 24 h	Air 72 h	Air 144 h	Water 144 h
Alanine	0.024±0.005	0.047±0.007[a]	0.052±0.004[a]	0.040±0.004	0.061±0.010[a]
Asparagine	0.018±0.002	0.035±0.002[a]	0.034±0.004[a]	0.036±0.001[a]	0.036±0.005[a]
Aspartate	0.002±0.001	0.003±0.001	0.006±0.001[a,b]	0.005±0.001[a]	0.003±0.001[c]
Arginine	0.026±0.003	0.030±0.005	0.021±0.003	0.019±0.001	0.029±0.002
Glutamine	0.202±0.089	0.374±0.019	0.688±0.028[a,b]	0.562±0.084[a]	0.169±0.014[c,d]
Glutamate	0.012±0.001	0.019±0.001	0.026±0.003	0.041±0.006[a,b,c]	0.017±0.002[d]
Glycine	0.045±0.005	0.135±0.022[a]	0.061±0.005[b]	0.147±0.018[a,c]	0.131±0.011[a,c]
Histidine	0.041±0.006	0.005±0.001[a]	0.005±0.001[a]	0.033±0.002[b,c]	0.033±0.001[b,c]
Isoleucine	0.058±0.008	0.004±0.001[a]	0.003±0.001[a]	0.004±0.001[a]	0.146±0.005[a,b,c,d]
Leucine	0.096±0.022	0.240±0.018[a]	0.208±0.007[a]	0.277±0.042[a]	0.264±0.007[a]
Lysine	0.017±0.003	0.040±0.003[a]	0.029±0.003	0.032±0.006	0.058±0.005[a,b,c,d]
Methionine	0.007±0.001	0.014±0.001[a]	0.013±0.001[a]	0.005±0.001[b,c]	0.011±0.001[d]
Phenylalanine	0.017±0.001	0.023±0.002	0.017±0.002	0.025±0.003	0.034±0.004[a,b,c,d]
Proline	0.003±0.001	0.011±0.001[a]	0.018±0.002[a,b]	0.024±0.002[a,b,c]	0.012±0.001[a,c,d]
Serine	0.009±0.001	0.017±0.001[a]	0.016±0.001[a]	0.020±0.003[a]	0.032±0.003[a,b,c,d]
Taurine	0.034±0.004	0.184±0.138[a]	0.049±0.011[b]	0.036±0.006[b]	0.028±0.004[b]
Threonine	0.048±0.007	0.047±0.002	0.056±0.004	0.067±0.002	0.072±0.006[a,b]
Tyrosine	0.014±0.002	0.444±0.028[a]	0.374±0.018[a]	0.024±0.003[b,c]	0.026±0.001[b,c]
Valine	0.070±0.015	0.255±0.019[a]	0.219±0.018[a]	0.208±0.027[a]	0.237±0.005[a]
TFAA	0.742±0.139	1.928±0.354[a]	1.893±0.426[a]	1.605±0.348[a]	1.398±0.194[b,c]

Results represent means ± S.E.M. (N=3).

[a]Significantly different from the value of 0 h, water control; [b]significantly different from the value of 24 h, air; [c]significantly different from the value of 72 h, air; [d]significantly different from the value of 144 h, air.

nitrogen (2615 μmol N) in the body of a 200 g *M. albus* became larger: −589 μmol N (Table 8). The deficit continued to increase with time, reaching −3516 (−5735 + 2219) μmol N at 144 h (Table 8). It is therefore logical to deduce that reductions in proteolysis and amino acid catabolism occurred when *M. albus* was exposed to long periods of terrestrial conditions. In this regard, *M. albus* is different from *O. marmoratus* (Jow et al., 1999) and *C. asiatica* (Chew et al., 2003b) but similar to *M. anguillicaudatus* (Chew et al., 2001) and *B. sinensis* (Ip et al., 2001b). *O. marmoratus* (Jow et al., 1999) and *C. asiatica* (Chew et al., 2003b) are apparently incapable of reducing proteolysis and amino acid catabolism during aerial exposure.

Reductions in proteolysis and amino acid catabolism constitute an effective strategy to slow down the internal accumulation of ammonia. If the rate of amino acid catabolism decreased while the rate of proteolysis remained unchanged, the steady-state concentrations of FAAs would increase.

Table 7. *Effects of 144 h aerial exposure on the activities of glutamine synthetase (GS), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutamate dehydrogenase (GDH) in the amination direction from the muscle, liver, brain and gut of* Monopterus albus

	Enzyme activity (μmol min^{-1} g^{-1} wet mass)							
	Muscle		Liver		Brain		Gut	
	Water	Air	Water	Air	Water	Air	Water	Air
GS	2.05±0.17	1.09±0.17*	25.37±2.08	36.60±4.32*	549.5±19.6	518.0±82.6	17.62±1.39	18.63±1.39
ALT	0.45±0.05	0.40±0.01	3.77±0.29	4.79±0.58	4.20±0.26	2.66±0.23*	1.00±0.05	1.47±0.05*
AST	3.57±0.44	4.76±0.28	30.19±0.99	39.76±2.49*	17.90±0.25	14.65±0.22*	13.14±0.65	13.55±0.94
GDH	0.10±0.02	0.06±0.01*	22.11±2.74	27.46±2.71	23.83±0.56	24.08±1.22	2.51±0.46	2.27±0.27

Results are means ± S.E.M. (N=4).

GS activity is expressed as μmol γ-glutamyl hydroxamate formed min^{-1} g^{-1} wet mass.

ALT, AST and GDH activities are measured in μmol NADH utilized min^{-1} g^{-1} wet mass.

*Significantly different from the corresponding control value.

Table 8. *Comparison of deficit in nitrogen excretion (μmol N) and credit in nitrogen accumulation (μmol N) in the muscle, liver, gut and plasma of a 200 g* Monopterus albus *exposed to terrestrial conditions for various times*

	24 h			72 h			144 h		
	Water	Air	Difference	Water	Air	Difference	Water	Air	Difference
Excreted from *M. albus* (200 g)									
Ammonia-N	1115	82	–1033	3341	257	–3084	6247	752	–5495
Urea-N	52	12	–40	152	32	–120	306	66	–240
Reduction in nitrogenous excretion			–1073			–3204			–5735
Retained in muscle (120 g)									
Ammonia-N	250	553	303	250	777	527	250	861	611
Urea-N	338	204	–134	338	224	–114	338	372	34
Glutamine-N	386	874	488	386	2426	2040	386	1828	1442
Retained in liver (3 g)									
Ammonia-N	14	30	16	14	46	32	14	42	28
Urea-N	4	2	–2	4	4	0	4	4	0
Glutamine-N	2	36	34	2	70	68	2	56	54
Retained in gut (5 g)									
Ammonia-N	7	19	12	7	19	12	7	23	16
Urea-N	2	8	6	2	8	6	2	4	2
Glutamine-N	8	8	0	8	20	12	8	16	8
Retained in plasma (8 ml)									
Ammonia-N	2	11	9	2	16	14	2	14	12
Urea-N	12	18	6	12	20	8	12	16	4
Glutamine-N	3	6	4	3	12	10	3	10	8
Increase in nitrogenous accumulation			724			2615			2219

However, if the rate of proteolysis decreased to a greater extent than the rate of amino acid catabolism, the steady-state levels of FAAs would decrease. In the case of *M. albus*, it would appear that the rate of proteolysis and the rate of amino acid catabolism were reduced proportionally because aerial exposure exhibited no significant effect on the TFAA content in the muscle, which is the bulk of the fish by mass, and other tissues.

The cells and tissues of M. albus *have high ammonia tolerance*

It is surprising that the ammonia concentrations in the tissues of *M. albus* built up to very high levels upon aerial exposure, reaching >7 μmol g^{-1}, >14 μmol g^{-1} and >3 μmol g^{-1} in the muscle, liver and brain, respectively (Fig. 2A–C). For mammals, a brain ammonia level of >1 μmol g^{-1} leads to encephalopathy. In mice, high ammonia levels in the brain induce an increase in extracellular glutamate, due to increased neuronal release, decreased re-uptake or both (Hilgier et al., 1991; Rao et al., 1992; Bosman et al., 1992; Schmidt et al., 1993; Felipo et al., 1994). It has been proposed that ammonia toxicity is mediated by excessive activation of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors in the brain (Marcaida et al., 1992), leading to cerebral ATP depletion (Marcaida et al., 1992; Felipo et al., 1994) and increases in intracellular Ca^{2+}, with subsequent increases in extracellular K^+ and cell death. Hermenegildo et al. (2000), however, showed that activation of NMDA receptors preceded the increase in extracellular glutamate. It had been suggested much earlier that NH_4^+ can substitute for K^+ and affect the membrane potential in the squid (*Loligo pealei*) giant axon (Binstock and Lecar, 1969). In addition, Beaumont et al. (2000) reported measured levels of depolarisation of muscle fibers in trout (*Salmo trutta*) with elevated levels of ammonia in their tissues (from –87 mV to –52 mV) that matched the effect predicted on the basis of the measured gradient for ammonium ions across the cell membranes. Thus, ammonia toxicity may be due to membrane depolarization and a rise in extracellular K^+ in the brain, exacerbated by NMDA receptor activation, glutamine-mediated astrocyte swelling, depletion of Krebs cycle intermediates and disruption of redox balance. These mechanisms are not mutually exclusive and could be additive in their effects. Besides *M. albus*, there are few fish that accumulate ammonia in their bodies to tolerate aerial exposure, as seen in the Indian catfishes (Saha and Ratha, 1998) and the weather loach (Chew et al., 2001; Tsui et al., 2002). The ammonia levels are not always evenly distributed within the fish; some exhibit much higher levels in the muscle compared with the brain whereas others can tolerate very high levels in the brain. How the cells and tissues of these animals tolerate these high ammonia levels is not clear at present. It is possible that they evolved to have special NMDA receptors, K^+-specific channels and K^+-specific Na^+/K^+-ATPase.

Conclusion

It can be concluded that the major strategies adopted by *M. albus* to deal with ammonia toxicity during 144 h of aerial exposure are (1) tolerance of ammonia at the cellular and subcellular levels, (2) detoxification of ammonia to glutamine and (3) reduction in ammonia production. The fact that ammonia and glutamine built up to high levels in the bodies of the experimental specimens by 144 h indicates that the fish might have already been stressed to a limit. Hence, it is unlikely that the same strategies would serve effectively to facilitate those *M. albus* that have to burrow into the mud and survive therein for long periods during drought. How *M. albus* deals with ammonia toxicity after burrowing into the mud awaits future studies.

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