In situ ammonia perfusion modifies the ureogenic potential of liver and kidney in the air-breathing fish, *Anabas testudineus* Bloch
4.1 Introduction

Ammonia, the principal nitrogenous waste product of amino acid breakdown in fish, is extremely toxic to animals including fish. Although fish seem to possess certain protective mechanisms against ammonia poisoning, they also excrete it directly and continuously into the environmental water via the gills without going through a detoxication process (Goldstein, 1982). The major portion of ammonia being excreted from the gills has been indicated as originating from the plasma which carries it there from the various tissues. The primary route of ammonia excretion in fish is through the gills (Wood, 1993; Wilkie, 1997). Waarde (1980) suggested that the blood ammonia might be produced mainly in the liver after food intake, whereas significant ammonia genesis may be taking place in the kidney and skeletal muscle under fasting and work loaded conditions. The presence of ornithine-urea cycle (OUC) enzymes in liver and kidney of climbing perch has reported (Saha and Ratha, 1989). Ammonia exposure in vivo, and in situ even at a very low concentration, is toxic to animals and therefore it either needs to be excreted promptly from the body or converted to other less-toxic molecules. Most teleost fishes are ammoniotelic, excreting ammonia as the major nitrogenous waste product by diffusion across the gills into the aqueous environment. Urea is a relatively less toxic and water-soluble molecule that requires lesser amount of water for its temporary storage and excretion.
In teleosts, the majority of nitrogenous waste is excreted through the gills as ammonia (Sayer and Davenport, 1987). When ammonia excretion was stimulated by infusion of ammonia, the increase in ammonia excretion rate was accompanied by an equivalent increase in sodium influx rate (Wilson et al., 1994). This tight coupling between ammonia excretion and sodium influx suggests that ammonium excretion occurs by Na⁺/NH₄⁺ exchange. It has been proposed (Evans, 1975a) that marine teleost fish extract Na from sea water in order to drive a Na/NH₄ and Na/H ionic exchange system which functions in nitrogenous waste excretion and acid-base regulation. A shift from ammonotelism to ureotelism can be seen in climbing perch during air-exposure (Ramaswamy and Reddy, 1983), though many air-breathing fish are ammonotelic (Saha et al., 1989). It appears that some air-tolerant fishes, such as the mudskippers *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*, reduce proteolysis and amino acid catabolism on land, particularly when held under constant darkness (Lim et al., 2001). When terrestrial conditions were accompanied by a natural photoperiod, in *P. schlosseri*, elevated free amino acid (FAA) concentrations in their muscle tissue (Ip et al., 1993, 2001). Ip et al (1993, 2001) reported that alanine levels were elevated in the tissues of air-exposed active *P. schlosseri*. The key advantage of partial amino acid catabolism is that ATP is produced without the net release of ammonia (Ip et al., 2001). A few species of fish continue to excrete primarily ammonia when out of water as...
in Blennius pholis (Davenport and Sayer, 1986). In the terrestrial environment, the absence of gill ventilation will presumably result in a decreased reliance on the gills and an increased reliance on the skin or kidneys for nitrogen excretion. Ammonia elimination via NH₃ volatilization has been documented in B. pholis, but accounts for only a small percentage (approximately 8%) of the total nitrogen excreted (Davenport and Sayer, 1986). Ammonia is highly soluble in water and permeates cell membrane relatively in an easy way. Water availability is clearly an important factor in the mode of nitrogen excretion. For instance; some crabs (Greenaway and Nakamure, 1991; De Vries and Wolcott, 1993) and isopods (Wieser and Schweizer, 1970; Wright and O' Donnel, 1994) excretes a significant portion of their nitrogen waste by ammonia volatilization.

Lim et al (2004) reported that when NH₄HCO₃ (10 μmol per g fish) was injected into the peritoneal cavity of swamp eel (Monopterus albus), the level of ammonia increased in the body. Acute ammonia toxicity in rainbow trout (Salmo gairdneri) was studied by intra-arterial injection of NH₄Cl and NH₄HCO₃ (Hillaby and Randall, 1979). Nile tilapia (Oreochromis niloticus) were infused with ammonium salts, acid, and base to investigate the effects of changes in arterial plasma total ammonia content and pH on plasma urea-nitrogen levels and urea-N excretory fluxes (David et al., 1999). Ip et al (2005) injected NH₄Cl intra-peritonealy into the African lungfish, Protopterus dolloi and reported that ammonia was excreted directly rather than converted to urea.
When NH4Cl was infused into the foot muscle of a snail, *Achatina fulica*, no increase in ammonia content was observed in the tissues but urea accumulation was observed in snails infused with NH4Cl. Hiong et al (2005) suggesting that urea synthesis in *A fulica* arose as primary response to ammonia detoxification. Michele and Wood (2009) reported that tissue levels of Rhesus (Rh) mRNA in rainbow trout (*Oncorhynchus mykiss*) changed when infused with NH4HCO3. The swamp eel, *Monopterus albus* when injected with NH4HCO3, the level of ammonia in the tissues elevated, suggesting that this fish have high tolerance of ammonia toxicity at the cellular and sub-cellular levels (Lim et al., 2004).

We studied the short-term *in situ* effect of ammonia perfusion and effects of ambient salinity on the ammonia handling by kidney and liver of air breathing fish. We analysed the indices of nitrogen metabolism and excretion after perfusion of varied ammonia concentration in freshwater and salinity-acclimated fish.

**4.2 Materials and methods**

Adult climbing perch, *Anabas testudineus* (35 ± 5 g body mass) collected from a local supplier were maintained in the laboratory in glass tanks. Fish were acclimated to tap water at 28 ± 2°C under natural photoperiod (12L/12D)
for two weeks prior to the experiment. Fish were fed with commercial feed at the rate of 1-5% of body mass/day.

Experimental protocol

Two independent experiments were carried out. In the first experiment varied concentrations of ammonia were infused into freshwater (FW) fish and in the second experiment used dilute seawater acclimated (SA; 20g L⁻¹) fish were used for ammonia perfusion. In the first experiment twenty-four FW fish were grouped into four of six each and kept in 60 L glass tanks. All the fish were anesthetized briefly in 0.1 % 2-phenoxyethanol (Sigma, St. Louis). These fish were placed on wet towels and an incision was made on the ventral side and heart was exposed. Polyethylene tubing was inserted into the bulbus arteum and perfusion medium was perfused at a rate of 1 ml/ min for 20 min as described earlier (Babitha and Peter, 2010). The first group of fish was the control which received the infusion medium only. Fish of 2, 3 and 4 groups were infused with varied doses of ammonium sulphate (0.75, 1.5 and 3μM) in a medium containing 119 mM NaCl, 5mM NaHCO₃, 5.4 mM KCl, 0.35 mM Na₂HPO₄, 0.81 mM MgSO₄, 1.25 mM CaCl₂, 5 mM D-Glucose for 20 minutes.

In the second experiment another batch of twenty-four SA fish were grouped into four of six each and kept in 60 L glass tanks. Group 1 fish held at
20 g L\(^{-1}\) seawater was the seawater control. Fish of 2, 3 and 4 groups were infused with 0.75, 1.5 and 3 \(\mu M\) ammonium sulphate for 20min.

After perfusion, kidney and liver were excised and kept at \(-20^\circ C\) and the ammonia, urea contents and the specific activity of ureogenic enzymes were quantified.

*Ammonia and urea estimations*

Liver and kidney urea content was estimated by DAM method using commercially available kit (Span Diagnostics, New Delhi). Deproteinised (Perchloric acid) and neutralized tissue samples were used for estimations. Ammonia was estimated according to the method of Bergmeyer and Beutler (1985). The ammonia generated in the samples was converted completely to glutamate by the enzyme GDH in the presence of \(\alpha\)-glutarate and NADH. The assay mixture contained 0.66 \(\mu M\) Tris, 0.2 \(\mu M\) \(\alpha\)-keto glutarate, 4 \(mM\) NADH, 1 \(\mu M\) ADP. The NADH oxidation which is equivalent to the amount of ammonia present in the sample was measured at 339nm using spectrophotometer (Systronics 2210, New Delhi) and the ammonia content was expressed as \(\mu M g^{-1}\) in wet tissue.

*Mitochondrial isolation*

Liver and kidney samples were homogenized in glycerol buffer \([20 mM Na_2HPO_4, 0.5 mM EDTA and 2 mM DTT dissolved in 50% glycerol in\]
Liver and kidney mitochondria were isolated by differential centrifugation (Plasto craft-Superspin-R) at 4°C and mitochondrial fraction was collected. The purity of mitochondrial fraction was checked by succinate dehydrogenate assay which showed agreeable results. Specific activity of arginase (ARG) ornithine transcarbomylase (OTC) and glutamate dehydrogenase (GDH) in the mitochondrial fraction and the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in the post-mitochondrial fraction.

**Arginase activity**

Arginase (E.C.3.5.3.1) catalyses the hydrolysis of L-arginine into ornithine and urea and is the terminal step of ornithine urea cycle (Dkhar et al 1991). Arginase activity was estimated spectrophotometrically by measuring the formation of urea from arginine as described by Polez et al (2003). The assay mixture contained 50 mM HEPES (pH 10), 250 mM L-Arginine and 10 mM MnCl₂ and a suitable volume of enzyme to reach a final volume of 1.5ml. The reaction was initiated by adding mitochondrial protein and incubated at 37°C for 5 min. The reaction was terminated with 70% TCA and urea produced was measured with the help of urea standards (30mg dL⁻¹). The arginase activity was expressed as μM hr⁻¹mg protein⁻¹.
Ornithine transcarbomylase activity

Ornithine transcarbamoylase (OTC; E.C.2.1.3.3) (also called ornithine carbamoyltransferase) is an enzyme that catalyzes the reaction between carbamoyl phosphate and ornithine to form citrulline and phosphate (P$_i$). OTC activity was quantified spectrophotometrically according to the method of Polez et al (2003). The assay mixture contained 50 mM HEPES (pH 8.5), 10 mM ornithine and 10 mM carbamyl phosphate and incubated with mitochondrial protein at 37°C for 15 min. The reaction was terminated by 70% TCA, and the citrulline formed was measured at 464nm (Rehmatullah and Boyde, 1980). The OTC activity was expressed as μM hr⁻¹mg protein⁻¹.

Glutamate dehydrogenase activity

Glutamate dehydrogenase (GDH; E. C.1.4.1.3) catalyses the conversion of ammonia and α-keto glutarate to glutamate (Das et al., 1991) which can be transmitted into different non-essential amino acids (FAA). GDH activity was measured spectrophotometrically according to the method of Danulat and Kempe (1992). The reaction mixture in a final volume of 3 ml contained 50 mM HEPES (pH 7.4), 250 mM NH$_4$Cl, 0.1 mm EDTA, 1 mM ADP, 14 mM α-keto glutarate, 0.12 mM NADH. The reaction was initiating by adding mitochondrial protein and the amount of NADH oxidized was taken as equivalent to the specific activity of GDH in the sample. Change in absorbance at 340 nm was
measured and specific activity of the enzyme was expressed as $\mu M$ hr$^{-1}$mg protein$^{-1}$.

**Alanine aminotransferase activity**

Alanine aminotransferase (ALT; E. C 2.6.1.2.), formerly known as glutamate pyruvate transaminase (GPT), is a pyridoxal enzyme, which catalyzes reversible transamination between alanine and 2-oxoglutarate to form pyruvate and glutamate. The ALT activity was measured spectrophotometrically according to the method of Reitman and Frankel (1957). The 0.5 ml reaction mixture contained 200mM alanine, 2mM $\alpha$-keto glutarate and cytosolic protein and incubated for 30min. The reaction was terminated with 2, 4 DNPH. The pyruvate produced during transamination as brown colored hydrazone, was measured at 505nm. And the activity was expressed as $\mu M$ hr$^{-1}$ mg protein$^{-1}$.

**Aspartate aminotransferase activity**

Aspartate transaminase (AST; E. C.2.6.1.1) is also called glutamic oxaloacetic transaminase (GOT). It facilitates the conversion of aspartate and $\alpha$-ketoglutarate to oxaloacetate and glutamate and vice-versa. The AST activity was measured spectrophotometrically according to the method of Reitman and Franken (1957). The 0.5 ml reaction mixture contained 200mM aspartic acid, 2mM $\alpha$-keto glutarate and cytosolic protein. After 30min of incubation the
reaction was terminated with 2, 4 DNPH. The pyruvate produced was measured at 505 nm and expressed as \( \mu M \text{hr}^{-1} \text{mg protein}^{-1} \).

**Statistics**

Data were collected from six animals in each group and are presented as mean ± standard error of the mean (s. e. m). All data sets were analyzed using one-way analysis of variance (ANOVA) followed by a pos-hoc multiple (all-pair-wise) SNK comparison test. Significant differences between groups were accepted if \( P<0.05 \) and all the statistical tests were performed using a software package (Graphpad Instat-3, San Diego, USA).

**4.3 Results**

**4.3.1 Effects of ammonia perfusion on FW fish**

*In situ* perfusion of ammonia in FW fish decreased the liver ammonia levels at 1.5 \( \mu M \) \( (P<0.01) \) and 3 \( \mu M \) \( (P<0.001) \) (Table 4.1). In this fish urea content in liver \( (P<0.01) \) increased with increasing concentration of ammonia perfusion but the kidney urea level remained unaffected (Table 4.1). The specific activity of arginase remained unaffected in the liver of FW fish after ammonia perfusion (1.5 \( \mu M \)) but showed a rise \( (P<0.01) \) in its activity in kidney after perfusion of ammonia (Fig 4.1A). The OTC activity in the liver of FW fish remained unaffected when infused with ammonia (Fig 4.2A). But the kidney
Fig. 4.1 Arginase specific activity in the liver and kidney of fresh water (FW) (Fig 1A) and dilute seawater acclimated (SA; 20 g L⁻¹) (Fig 1B) climbing perch after infusion with 0.75, 1.5 and 3µM ammonia. Each column represents mean ± SEM for six fish. *(P<0.05), ** (P<0.01) and *** (P<0.001) show significant difference when compared with control (FW or SA).
Fig. 4.2 OTC specific activity in the liver and kidney of fresh water (FW) (Fig 2A) and dilute sea water acclimated (SA; 20 g L⁻¹) (Fig 2B) climbing perch after infusion with 0.75, 1.5 and 3 μM ammonia. Each column represents mean ± SEM for six fish. **(P<0.01) and *** (P<0.001) show significant difference when compared with control (FW or SA).
Fig. 4.3 GDH specific activity in the liver and kidney of fresh water (FW) (Fig 3A) and dilute seawater acclimated (SA; 20g L⁻¹ (Fig 3B) climbing perch after infusion with 0.75, 1.5 and 3μM ammonia. Each column represents mean ± SEM for six fish### (P<0.001) show significant difference when compared compared with FW control. *(P<0.05), ** (P<0.01) and *** (P<0.001) show significant difference when compared with control (FW or SA).
Table 4.1
Ammonia and urea contents in liver and kidney (μmol g wet tissue⁻¹) of freshwater (FW) and dilute seawater-acclimated (SA; 20 g L⁻¹) climbing perch perfused with 0.75, 1.5 and 3 μM ammonia. Each value is mean ± SEM for six fish.

<table>
<thead>
<tr>
<th>Status</th>
<th>0</th>
<th>0.75</th>
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<th>3.0</th>
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<td><strong>Ammonia in FW fish</strong></td>
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<tr>
<td>Liver</td>
<td>14.0 ± 1.1</td>
<td>11.6 ± 1.3</td>
<td>7.9 ± 1.0**</td>
<td>4.9 ± 0.5***</td>
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<tr>
<td>Kidney</td>
<td>6.7 ± 1.2</td>
<td>6.0 ± 0.6</td>
<td>9.8 ± 0.3</td>
<td>4.8 ± 0.6</td>
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<tr>
<td><strong>Ammonia in SA fish</strong></td>
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<tr>
<td>Liver</td>
<td>7.6 ± 1.2</td>
<td>8.2 ± 0.6</td>
<td>10.6 ± 0.6</td>
<td>10.6 ± 2.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>10.3 ± 1.2</td>
<td>8.9 ± 1.2</td>
<td>9.8 ± 1.2</td>
<td>11.2 ± 1.2</td>
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<td><strong>Urea in FW fish</strong></td>
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<tr>
<td>Liver</td>
<td>6.4 ± 0.5</td>
<td>2.6 ± 0.5**</td>
<td>3.4 ± 0.3**</td>
<td>3.2 ± 0.3**</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.9 ± 1.1</td>
<td>7.9 ± 0.8</td>
<td>7.8 ± 0.6</td>
<td>6.6 ± 1.3</td>
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<tr>
<td><strong>Urea in SA fish</strong></td>
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<tr>
<td>Liver</td>
<td>11.1 ± 0.4</td>
<td>20.8 ± 3.1*</td>
<td>15.5 ± 0.8</td>
<td>42.9 ± 5.0***</td>
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<tr>
<td>Kidney</td>
<td>6.0 ± 0.4</td>
<td>5.5 ± 0.4</td>
<td>11.8 ± 0.3***</td>
<td>18.5 ± 1.0***</td>
</tr>
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</table>

*(P<0.05), ** (P<0.01) and*** (P<0.001) show significant difference when compared with control (FW or SA).

OTC activity decreased (P<0.001) at higher doses (1.5 and 3 μM) of ammonia perfusion (Fig 4.2A). The specific activity of GDH in the liver of FW fish increased after ammonia perfusion (Fig 4.3A). The highest GDH activity was noticed at low (0.75 μM) concentration of ammonia (P<0.001). In the kidney of FW fish no significant change in the GDH activity observed after perfusion of ammonia (Fig 4.3A).

The activity of liver AST of FW fish increased (P<0.05) at low (0.75 μM) and high (3 μM) concentrations of ammonia perfusion. The activity of
ALT in the liver of FW fish increased \((P< 0.01)\) at all concentrations (0.75, 1.5 and 3 \(\mu M\)) of ammonia perfusion without any effect on kidney (Table 4.2).

Table 4.2
AST and ALT specific activities (\(\mu \text{mol hr}^{-1} \text{ mg protein}^{-1}\)) in the liver and kidney of freshwater (FW) and dilute seawater-acclimated (SA; 20 g L\(^{-1}\)) climbing perch perfused with 0.75, 1.5 and 3 \(\mu M\) ammonia. Each value is mean ± SEM for six fish.

<table>
<thead>
<tr>
<th>Status</th>
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<th>0.75</th>
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<tr>
<td><strong>AST in FW fish</strong></td>
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<tr>
<td>Liver</td>
<td>6.7 ± 0.4</td>
<td>13.1 ± 1.3*</td>
<td>8.4 ± 0.5</td>
<td>14.2 ± 1.8*</td>
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<tr>
<td>Kidney</td>
<td>6.2 ± 0.5</td>
<td>5.9 ± 0.4</td>
<td>6.1 ± 0.2</td>
<td>5.2 ± 0.5</td>
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<tr>
<td><strong>AST in SA fish</strong></td>
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<tr>
<td>Liver</td>
<td>14.5 ± 0.24</td>
<td>12.8 ± 0.5</td>
<td>16.6 ± 2.3</td>
<td>16.4 ± 0.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>9.4 ± 0.6</td>
<td>7.6 ± 0.6</td>
<td>7.3 ± 0.6</td>
<td>10.1 ± 1.2</td>
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<tr>
<td><strong>ALT in FW fish</strong></td>
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</tr>
<tr>
<td>Liver</td>
<td>14.9 ± 1.56</td>
<td>25.6 ± 2.3**</td>
<td>29.2 ± 2.3**</td>
<td>24.2 ± 1.2**</td>
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<tr>
<td>Kidney</td>
<td>16.8 ± 1.2</td>
<td>17.6 ± 0.5</td>
<td>16.2 ± 1.3</td>
<td>17.0 ± 2.0</td>
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<tr>
<td><strong>ALT in SA fish</strong></td>
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<td></td>
</tr>
<tr>
<td>Liver</td>
<td>19.4 ± 1.8</td>
<td>18.4 ± 1.8</td>
<td>25.7 ± 1.8*</td>
<td>20.7 ± 1.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>26.7 ± 1.5</td>
<td>17.4 ± 1.2*</td>
<td>21.0 ± 1.2</td>
<td>31.2 ± 3.0</td>
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</tbody>
</table>

\(*(P<0.05)\) and \(** (P<0.01)\) show significant difference when compared with control (FW or SA).

4.3.2 Effects of ammonia perfusion on SA fish

In SA fish the ammonia content in the liver and kidney remained unaffected after ammonia perfusion (Table 4.1). The urea content showed an elevation at low \((P<0.05)\) (0.75 \(\mu M\)) and high \((P<0.001)\) (3 \(\mu M\)) doses of ammonia.
perfusion in liver and kidney (Table 4.1). In SA fish the activity of arginase increased ($P<0.05$) in the liver and kidney after ammonia perfusion at 1.5 $\mu M$ and 3 $\mu M$ concentration (Fig 4.1B). Perfusion of ammonia at 1.5 $\mu M$ increased OTC ($P<0.01$) in the kidney but without any effect on liver (Fig 4.2B). Ammonia perfusion declined ($P<0.001$) the GDH activity in a dose dependent manner (Fig 4.3B).

The AST activity in the liver and kidney of SA fish was not affected by ammonia perfusion (Table 4.2). The ALT activity in the liver and kidney of SA fish showed increasing ($P<0.05$) after 1.5 $\mu M$ ammonia perfusion (Table 4.2).

4.4 Discussion

Fresh water fish when infused with ammonia showed reduction in the urea and ammonia contents in their tissues. This indicates that FW liver could easily handle exogenous ammonia. The unaffected kidney urea after ammonia loading also supports that FW fish can easily tolerate ammonia toxicity probably with the help of liver and not with the kidney. In contrast to FW fish, the SA fish kidney showed an increase in urea production pointing to the possibility of ureogenic induction by this organ. Ammonotelism is the primary method utilized by most aquatic invertebrates (Loong et al., 2002). Ammonia is stored in the body of fish in high concentrations relative to basal excretion rates (Randall and Wright, 1987). Most teleost fish are ammoniotelic and excrete
ammonia as their principal waste product (Wilkie, M., 1997). Mckenzie et al (1999) reported that when Nile tilapia (*Oreochromis niloticus*) were infused with 200 mM NH₄Cl increased ammonia, reduced pH, and increased plasma urea-N and urea-N excretion. Saha et al (1995) studied the induction pattern of urea cycle enzymes and the rate of urea–N excretion by perfusing different concentrations of ammonium chloride in the liver of Asian Stinging catfish (*Heteropneustes fossilis*). They reported that *H. fossilis* have the capacity of transition from ammoniotelism to ureotelism to tolerate very high ambient ammonia.

The diminished capacity of FW fish to retain ammonia in the liver and kidney also suggest that our FW fish is not tuned to utilize ammonia for synthesizing urea. Similar result has been obtained in African lungfish, *Protopterus dolloi* which showed ammonia excretion rather than the induction of ureogenesis when injected with ammonia Ip et al (2005). They suggest that the injected ammonia was likely to stay in extracellular compartments available for direct excretion. It is also possible that some of ammonia infused might have converted into non-essential amino acids. Studies on swamp eel *Monopterus albus* (Lim et al., 2004) with NH₄HCO₃, showed that the increased ammonia in this fish could be a compensatory adaptation to remove its majority of exogenous ammonia. The similar rise in the glutamine content in the brain of
this fish at hour 6 also confirms the capacity of swamp eel to detoxify ammonia through glutamine synthesis.

It is possible to consider that the detoxification pathways exist in the body might be sufficient to detoxify extra ammonia. Alternatively it appears that the lowered ammonia and urea levels may be due to induction of urea cycle enzymes in this fish. Hillaby and Randall (1979) studied acute ammonia toxicity in rainbow trout (Salmo gairdneri) by intraarterial injection of NH₄Cl and NH₄HCO₃ and reported that there was rapid excretion of ammonia followed by the infusion.

Saha et al. (2002) reported that the walking catfish when exposed to 25 mM NH₄Cl over a period of seven days caused an increase in the concentration of various nonessential FAAs in different tissues by two- to threefold. The main amino acids whose concentrations increased were glutamine, glutamate, aspartate, alanine, glycine, and taurine. This was accompanied by induction of the activity of certain amino acid metabolism-related enzymes including GDH, AST, and ALT. In our study the increase in AST and ALT activities suggest that FW fish have an enhanced ability to utilize alanine and aspartate metabolites. Likewise, the elevated GDH activity in FW fish also supports the detoxification of ammonia mainly by converting it into glutamate. Formation of glutamine has been supposed to be the most efficient way of removing ammonia from tissues (Zakim, 1981). Glutamine has been found to play a role in ammonia
detoxification in fish in response to high environmental ammonia
centrations (Arillo et al., 1981; Dabrowska and Wlasow, 1986; Mommsen
and Walsh, 1992; Peng et al., 1998; Tsui et al., 2002). The weather loach
*Misgurnus anguillicaudatus* when loaded with ammonia, there was no
accumulation of glutamine (Tsui et al., 2002). As the internal ammonia levels
increased, *M. anguillicaudatus* was able to excrete some ammonia in the gaseous
form (NH₃).

On the contrary, in the SA fish the rise in urea in the kidney and liver
clearly document induction of ureogenic capacity of these organs. Furthermore,
this ability of SA fish to detoxify ammonia is dose-dependent as the induction
of urea cycle enzyme increases with increasing concentration of ammonia. For
example the urea content in the kidney of SA fish increased after 1.5 μM
ammonia perfusion. It is interesting to see that lower concentration of ammonia
was sufficient enough to initiate maximum induction of urea cycle enzymes in
this fish. Saha et al. (1995) reported that higher ammonia load in the liver
beyond a threshold level causes enhanced ureogenesis via an induced urea
cycle. Such ammonia- induced ureogenesis has also been reported in ureotelic
amphibians (Janssens and Cohen, 1968; Janssens, 1972). Our results also
confirm that upon salinity exposure climbing perch acquire the capacity to
switch over to ureotelism from ammoniotelism which later help them to
tolerate ammonia toxicity.
Overall our *in situ* data support the hypothesis that airbreathing fish can tolerate ammonia toxicity either by relying on aminoacid chanelling that occur in FW fish by promoting ureogenic potential in the fish acclimated to salinity.