ELSEVIER

Comparative Biochemistry and Physiology Part A 120 (1998) 195–208

CBP

Review

Ureogenesis in Indian air-breathing teleosts: adaptation to environmental constraints

N. Saha [a,*], B.K. Ratha [b]

[a] *Biochemical Adaptation Laboratory, Department of Zoology, North-Eastern Hill University, Shillong, 793022, India*
[b] *Environmental Biochemistry Laboratory, Centre for Advance Study in Zoology, Banaras Hindu University, Varanasi, 221005, India*

Received 28 October 1996; received in revised form 24 October 1997; accepted 20 January 1998

Abstract

Most of the Indian air-breathing teleosts are primarily ammoniotelic, but appear to have retained the genes for the urea cycle enzymes, since a full complement of urea cycle enzymes have been reported for many of them. The ability to synthesize urea by these fish is probably due to their amphibious nature, and their normal habitat of swamps, where the water ammonia level may to be quite high, is uninhabitable to any typical freshwater teleosts. One of these air-breathing species, the singhi catfish (*Heteropneustes fossilis*), can tolerate very high ambient total ammonia concentrations (up to 75 mM ammonium chloride) for weeks without any deleterious effects. Transition from ammoniotelism to ureotelism occurs in some of these species of air-breathing fish when exposed to apparently stressful conditions such as higher ambient ammonia, to air, and also when they live in semidry condition inside mud during habitat drying. Although the real mechanism(s) of regulation of ureogenesis is not clear in these fish, given available data, it is hypothesized that the accumulation of ammonia within the body per se under the above stressful conditions is likely the internal modulator for enhanced ureogenesis mainly to avoid any build up of ammonia to a level that can be toxic to these fish. An active urea cycle is believed to predominate over uricolysis as a source of urea, even though both pathways are present in these air-breathing fish. The presence of significant levels of both carbamyl phosphate synthetase (CPS), CPS I-like and CPS III activities, reported in some air-breathing catfishes, may represent intermediate scenarios for a proposed evolutionary transition from CPS III to CPS I, or may play an important physiological adaptive role in the tolerance of these fish to high concentrations of ambient ammonia

Keywords: Ammonia; Urea; Urea cycle; Ammoniotelism; Ureotelism; Ureogenesis; Hyper-ammonia stress; Aerial exposure; Air-breathing teleosts; *Heteropneustes fossilis*; Environmental constraints; Amphibious

1. Introduction

Nitrogen metabolism is considered to be one of the most sensitive physiological systems showing adaptive responses to environmental variations. Accordingly, the nature of major nitrogen excretory products in animals has changed with the evolution of vertebrates from water to the land habitat (for reviews, see Refs. [12,20,111]). Most teleost fishes excrete ammonia as the major nitrogenous product resulting from the catabolism of dietary or structural proteins and amino acids for the purpose of energy production [12,72,99]. However, one of the major problems faced by a majority of teleosts is the relative toxicity of ammonia when it is concentrated in blood and in body tissues. In general, however, aquatic animals can tolerate more elevated levels of blood ammonia than terrestrial animals. Plasma total ammonia ($NH_3 + NH_4^+$) normally remains between 0.05 and 2 mmol l^{-1} in most teleosts

* Corresponding author. Fax: +91 364 250076; e-mail: nsaha@nehus.ren.nic.in

1095-6433/98/$19.00
PII S1095-6433(98)00026-9

[13,108,113] with the exception of singhi catfish, where it has been reported at levels of up to 4 mmol l^{-1} in higher ambient ammonia concentrations [81]. In contrast, blood ammonia levels greater than 0.05 mmol l^{-1} can be toxic to the central nervous system of most mammals [21,60]. Ammonia is either excreted directly if feasible, or converted to some less toxic compounds such as urea, uric acid or amino acids in different animals [12].

Although the majority of teleost fishes are ammoniotelic, urea also constitutes about 10–30% of the total nitrogenous wastes in most of them (Table 1). But, as an exception, in marine elasmobranchs, urea constitutes the major nitrogen excretory product, and concentrations of urea as high as 0.4 M are maintained in blood and tissues for the purpose of osmoregulation [69]. The source of urea in teleosts and the involvement of the ornithine-urea cycle (hereafter referred to as urea cycle) are still under debate. Until recently, the presence of a functional urea cycle, which appears to be the major source of urea formation in higher vertebrates, was not known to exist in teleosts. The presence of a functional urea cycle has recently been reported in various teleosts, such as, in some Indian air-breathing teleosts [78,79], alkaline lake adapted tilapia, *Oreochromis alcalicus grahami* [73], and marine toadfishes, *Opsanus tau* and *O. beta* [62,75]. Accordingly, interest in the study of the urea cycle, the expression of the urea cycle during early embryonic developmental stages, regulation of expression of urea cycle enzymes, and nitrogen excretion patterns under different environmental constraints in different teleosts has recently increased.

Table 1
The rate of excretion of ammonia-N and urea-N in different groups of fishes

Species	Habitat	Ammonia-N (μmol kg^{-1} body wt h^{-1})	Urea-N (μmol kg^{-1} body wt h^{-1})	Ratio of ammonia-N:urea-N	References
Purely aquatic:					
Cyprinus carpio (carp)	FW	330	30	11.0	[90]
Carassius auratus (gold fish)	FW	194	30	6.5	[90]
Oreochromis nilotica (tilapia)	FW	763	130	5.5	[109]
Onchorhynchus mykiss (rainbow trout)	FW	220	32	6.9	[103]
Platichthys stellatus (starry flounder)	SW	253	34	9.7	[107]
Liptocottus armatus (sculpin)	SW	122	32	3.6	[107]
Engraulis mordax (anchovy)	SW	293	71	4.2	[57]
Trachurus symmetricus (jack mackerel)	SW	271	50	5.4	[57]
Agonus cataphractus	SW	645	127	5.1	[86]
Cramlabrus melops	SW	372	18	21.0	[86]
Limanda limanda (yellow flounder)	SW	490	36	13.6	[86]
Opsanus beta (Gulf toad fish)	SW	75	22	3.4	[101]
Oreochromis alkalicus grahami (tilapia)	Lake Magadi (pH 10) 50% ~ SW	0	7600	—	[73,101]
Onchorhynchus clarki henshawi (Lahontan cutthroat trout)	Pyramid lake (pH 9.4) ~10% SW	123	63	2.0	[112]
Chalcalburnus tarichi (tarek)	Lake Van (pH 9.8)	1041	607	1.7	[25]
Air-breathing					
Amphipnous cuchia (cuchia eel)	FW	171	34	5.0	[79]
Clarias batrachus (walking catfish)	FW	421	74	5.7	[79]
Heteropneustes fossilis (singhi catfish)	FW	254	48	5.3	[79]
Anabas testudineus (climbing perch)	FW	417	32	13.0	[79]
Anabas scandens (climbing perch)	FW	921	600	1.5	[70]
Channa punctatus (snake head)	FW	585	71	16.0	[79]
Channa gachua (snake head)	FW	594	700	0.9	[70]
Mystus vitatus (bagrid catfish)	FW	883	560	1.6	[70]
Amia calva (bow fin)	FW	607	60	10.1	[59]
Blennius pholis (blenny)	FW	93	22	4.2	[27]
Sicyases sanguineus (mudskipper)	BW	270	760	0.4	[38]
Periophthalmus sobrinus (mudskipper)	40% SW	770	60	12.8	[36]
Periophthalmus cantonensis (mudskipper)	BW	630	1260	0.5	[39]
Periophthalmus cantonensis (mudskipper)	25% SW	445	305	1.5	[65]
Boliophthalmus pectinirostris(mudskipper)	25% SW	240	95	2.5	[65]

Standard errors are not listed. FW, freshwater; SW, seawater; and BW, brackish water.

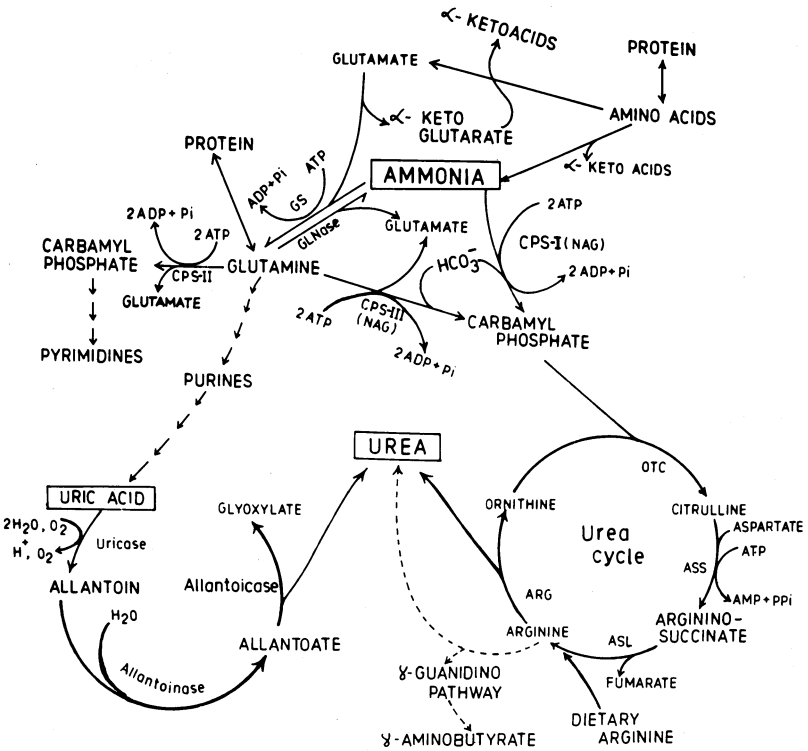

Fig. 1. Outlines of different possible pathways of urea synthesis in fish. CPS, carbamyl phosphate synthetase; OTC, ornithine transcarbamylase; ASS, argininosuccinate synthetase; ASL, argininosuccinate lyase; ARG, arginase; GDH, glutamate dehydrogenase; GS, glutamine synthetase; GLNase, glutaminase; NAG, *N*-acetyl-L glutamate

Air-breathing teleosts of the Indian subcontinent are unique among freshwater teleosts in possessing a very active urea cycle, the capacity to switch from ammoniotelism to ureotelism under hyperammonia stress and during exposure to air, and also for having the capacity to tolerate very high ambient ammonia [74,78–82]. This paper reviews ureogenesis and its role in allowing Indian freshwater air-breathing teleost species to thrive in environments normally considered unsuitable for other fishes. When appropriate, comparisons with ureogenesis of other teleosts are included.

1.1. Habitat of Indian freshwater air-breathing teleosts

Several species of freshwater air-breathing teleosts exist on the Indian subcontinent. All of them have accessory air-breathing structures (for reviews, see Refs. [30,66,67]), which are thought to have evolved as an adaptation to hypoxic water conditions during severe periodic droughts [52,71]. Obligatory air breathers include climbing perch (*Anabas testudineus*), cuchia eel (*Amphipnous cuchia*), two snakeheads (*Channa striata* and *Channa marulis*), and the facultative air-breathers include singhi catfish (*Heteropneustes fossilis*), walking catfish (*Clarias batrachus*), the two snakeheads (*Channa punctatus* and *Channa gachua*) [66]. Both obligatory and facultative air-breathers usually inhabit stagnant, slow flowing swampy water bodies or wet lands, which are usually uninhabitable to purely aquatic fishes such as carp. These swamps, which are often covered with macrovegetation like water hyacinth, are characterized by a low dissolved oxygen ($P_{O_2} = 2.5–30$ torr), a water pH range of 6.5–7.8, more free carbon dioxide gas (0.24–1.7 mmol l^{-1}), bicarbonate concentrations of 0.6–2.5 mmol l^{-1}, high ammonia levels of 2–15 mmol l^{-1} (evolved mostly as a degradable product of micro- and macrovegetation), and a water temperature of 20–30°C [18]. During summer, when the swamps dry up, fish face more adverse ecological conditions and most of the air-breathing fishes burrow inside mud to avoid total dehydration. Some of them, such as cuchia eel, live inside mud almost throughout the year. Among other things, an exceptional tolerance to high ambient ammonia and low ambient oxygen has made some of these species successful candidates for aquaculture. Under laboratory conditions these fishes have been shown to tolerate periods of total dehydration; cuchia eel (*A. cuchia*) survives for 90–100 h, singhi (*H. fossilis*) and walking catfish (*C. batrachus*) for 60–70 h, and climbing perch (*A. testudineus*) and one snakehead (*C. punctatus*) for 8–12 h [79].

1.2. Ureogenesis in air-breathing teleosts

The two major routes for urea synthesis in fish are believed to be: (i) uricolysis, i.e. the oxygen dependent

conversion of uric acid, derived from purine nucleotides, to urea; and (ii) arginolysis of arginine to urea by the enzyme arginase [22,23,31,41,64,104,105,110,113] (Fig. 1). In addition, there could be two other routes of urea production in fish such as: (iii) the urea cycle, reported recently in some teleosts; and (iv) the γ-guanidino urea hydrolase pathway (Fig. 1).

Like many other non-ureogenic teleosts, high levels of activity of all the uricolytic enzymes have been reported in the liver of air-breathing singhi catfish (*H. fossilis*), but it was suggested that the major part of urea production takes place in this fish mostly via the urea cycle [78]. In other Indian air-breathing species, the occurrence of the uricolytic pathway is yet to be established.

Activities of urea cycle enzymes were studied in at least five species of Indian air-breathing teleosts [78,79]. In four species (*H. fossilis*, *C. batrachus*, *A. cuchia* and *A. testudineus*) high levels of activity for all the five urea cycle enzymes could be detected in the liver. With the exception of *A. testudineus*, these species also possess the full complement of urea cycle enzymes in kidney tissue. The activity of argininosuccinate synthetase (ASS) could not be detected in the kidney of *A. testudineus*, and in neither the liver nor the kidney of *C. punctatus* [78,79]. The activities of all the enzymes were higher in the liver than in the kidney of the fish studied, except in the case of *A. cuchia*, where the kidney showed a higher level of activity. The observed activities of enzymes of the urea cycle in Indian air-breathing teleosts were higher than those in exclusively freshwater teleosts and showed similarities with aestivating lungfish, marine toadfish of the genus *Opsanus*, an aquatic amphibian, *Xenopus laevis*, and alkaline Lake Magadi tilapia, where the presence of the urea cycle has been confirmed (Table 2 for comparisons and Refs.). The ratios of excretion of ammonia: urea-N in Indian air-breathing teleosts were also found to be higher than for many other freshwater and marine teleosts (Table 1 for comparisons and Refs). However, all the air-breathing teleosts are primarily ammoniotelic, excreting ammonia mostly through extra-renal sources when they live in water [79,82] and becoming facultatively ureotelic when exposed to apparently stressful environmental conditions such as high ambient ammonia, exposure to air or in semidry condition during habitat drying (see below).

The subcellular localization of urea cycle enzymes, glutamine synthetase (GS), and also the types of urea synthesis-related carbamyl phosphate synthetase (CPS), CPS I or III, the first enzyme of urea cycle, vary in different groups of vertebrates and have been assigned some physiological and evolutionary significance. Uniquely high levels of GS present in the liver and kidney of singhi catfish are mainly localized in the mitochondria [17], which is analogous to uricotelic species [14,97,98], an obligatory ureotelic elasmobranch (*S. acanthias*) [16], and facultatively ureotelic gulf toadfish (*O. beta*) [5]. The subcellular distribution of the urea cycle enzymes in the liver (and kidney) of singhi catfish was found to be analogous to that of elasmobranchs and marine toadfish [29,84], i.e. the urea cycle-related glutamine- and *N*-acetyl-L-glutamate (NAG)-dependent CPS III, ornithine transcarbamylase (OTC), and arginase (ARG) are localized in the mitochondria, and argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) are localized in the cytosol. The co-localization of GS along with CPS III in mitochondria of this air-breathing catfish probably helps in the assimilation of ammonia to urea more efficiently. In contrast to elasmobranchs and like other teleosts, cytosolic pyrimidine-related CPS II (glutamine-dependent) is present in the liver (and kidney) of singhi catfish; the level of activity is reported to be higher than has been reported for other teleosts [84]. Given the known ureotelic capacity, the presence of CPS III activity in liver (and kidney) of singhi catfish almost at the level of few known ureotelic teleost species, i.e. in marine toadfish [5,62] and in alkaline lake-adapted tilapia [73] is not unexpected. However, the presence of mammalian and amphibian type of CPS I-like (ammonia- and NAG-dependent) activity at a level higher than CPS III in the mitochondria of both the liver and kidney of singhi catfish reported recently [84] is unique from that observed in other teleosts. Similar GS activity, types of CPSs and subcellular localization have also been observed in another air-breathing walking catfish (*C. batrachus*) (Saha, Das, Dutta and Dkhar, submitted). The presence of a gene for both a CPS III and a CPS I in these air-breathing catfishes would not seem to be a likely explanation for these two activities given current understanding of the structural relationships between these two enzymes, their species distributions (CPS I is present only in ureotelic mammalian and amphibian species, CPS III is present only in invertebrates and fish) and the prevailing view that CPS I evolved from CPS III [3,4,13,43,46,63]. Perhaps adaptation in these air-breathing catfishes and closely related species was achieved as a separate event in which the CPS III gene underwent duplication and one gene subsequently lost the structural requirements for utilization of glutamine as substrate. Alternatively, perhaps the observed CPS I-like activity represents an adapted form of CPS III with separate ammonia and glutamine binding sites. Either of these possibilities would represent possible intermediate scenarios for a proposed evolutionary transition from CPS III to CPS I. Elucidation of the nature of the two types of CPS activities will require purification and characterization of the enzyme(s) responsible for these activities and the mRNA(s) coding for the enzyme(s).

Table 2
Activities of urea cycle enzymes (μmol g^{-1} wet wt h^{-1}) in the liver of different groups of fishes and aquatic amphibia

Species	Urea synthesis via urea cycle	CPS (assayed in the presence of ammonia + NAG)	CPS (assayed in the presence of glutamine + NAG)	OTC	ASS	ASL	ARG	References
(a) Freshwater teleosts								
Cyprinus carpio (carp)	No	0.42	—	1.0	0.5	0.4	58	[48]
Salmo gairdneri (rainbow trout)	No	0.13	—	1.4	1.7	1.1	1326	[48]
Salmo gairdneri (rainbow trout)	No	2.6	—	0.5	—	—	5778	[19]
Carassius auratus (goldfish)	No	0.09	—	—	2.8	BLD	71	[48]
Scardinus erithrophthalmus (mud)	No	0.22	—	—	3.6	0.2	170	[48]
Rutilus rutilus (roach)	No	0.11	—	1	0.3	0.8	329	[48]
Tinca vulgaris (tench)	No	0.19	—	2.4	3.6	1.3	366	[48]
Siluris glanis (catfish)	No	BLD	—	—	9.4	0.3	3542	[48]
Perca fluviatilis(perch)	No	0.04	—	4	BLD	1.4	990	[48]
Anguilla anguilla (eel)	No	0.16	—	6	1.6	1.8	4565	[48]
Ictalurus punctatus (channel catfish)	No	2.97	—	221	BLD	—	5472	[106]
Micropterus salmoides (largemouth bass)	No	—	0.6	126	—	—	2340	[15]
(b) Freshwater air-breathing teleosts								
Heteropneustes fossilis (singhi catfish)	Yes	4.6	2.11	252	28.5	27.1	7699	[78,84]
Clarias batrachus (walking catfish)	Yes	2.4	—	312	13	21.9	6654	[79]
Amphipnous cuchia (cuchia eel)	Yes	1.2	—	93	23.4	16.4	597	[79]
Anabas testudineus (climbing perch)	Yes	2.8	—	113	19.7	12.8	3580	[79]
Channa punctatus (snake head)	No	2.1	—	78	BLD	6.1	2889	[79]
(c) Freshwater alkaline adapted teleosts								
Oreochromis alcalicus grahami (tilapia)	Yes	—	1.24	439	—	—	703	[73]
Oncorhynchus clarki henshawi (Lahontan cutthroat trout)	No	—	0.02	1.8	2.4	—	2458	[104]
Catostomus tahoensis (tahoe sucker)	No	—	1.5	0.3 6	4.9	—	263	[58]
Gila bicolor (tui chub)	No	—	0.42	0.7 2	3.9	—	311	[58]
(d) Marine teleosts								
Opsanus tau (oyster toadfish)	Yes	0.91	3.7	2418	—	—	25 026	[62]
Opsanus beta (Gulf toadfish)	Yes	0.84	7.8	3127	4.8	19.8	1889	[62]
Porichthys notatus (plainfin midshipman)	No	—	1.2	606	2.4[b]	2.4[b]	504	[5]
Clupea harengus (herring)	No	0.23	—	1.7	BLD	1.2	664	[48]

Table 2 (Continued)

Species	Urea synthesis via urea cycle	CPS (assayed in the presence of ammonia + NAG)	CPS (assayed in the presence of glutamine + NAG)	OTC	ASS	ASL	ARG	References
Mullus barbatus (red mullet)	No	0.24	—	179	3.4	1.7	714	[48]
Salmo salar (Atlantic salmon)	No	0.03	—	4	0.6	0.7	1117	[48]
Elasmobranchs								
Dasyatis americana (freshwater stingray)	Yes	6.5	—	8450	21.6	—	34 800	[32]
Urolophus jamaicensis (round ray)	Yes	4.5	—	8450	16.5	—	13 920	[32]
Potamotrygon sp. (freshwater stingray)	Yes	0.35	—	1600	9.4	—	4310	[32]
Squalus acanthias (dogfish shark)	Yes	—	18	198	36	42	648	[16]
Lungfishes								
(a) Purely aquatic *Neoceratodus forsteri*	No	0.3	—	154	4.3	8.5	1048	[34]
(b) Aestivating *Protopterus aethiopicus*	Yes	31.2	—	1675	6.6	56.8	3480	[34]
Coelacanth								
Latimeria chalumnae	Yes	4.75	—	8240	10	—	30 000	[33]
Aquatic amphibia								
Xenopus laevis	Yes	13	—	77	11	37	9943	[48]

Standard errors are not listed. —, not assayed; BLD, below the level of detection; CPS, carbamyl phosphate synthetase; OTC, ornithine transcarbamylase; ASS, argininosuccinate synthetase; ASL, argininosuccinate lyase; ARG, arginase; NAG, *N*-acetyl-L-glutamate.

[b] Both ASS and ASL were measured together.

All Indian air-breathing teleosts are oviparous and deposit their eggs in the mud at the bottom of swamps for fertilization and development. During the early embryonic development and also at different stages of development after hatching, these fish must be protected from ammonia toxicity generated either endogenously by the catabolism of yolk protein or from the external ammonia already present at high levels. The early developmental stages might be more sensitive to ammonia toxicity than the adult. Therefore, there should be mechanism(s) for detoxification of the accumulated ammonia in these air-breathing teleosts during the early life stages, one of which could be the presence of a more active urea cycle than the adult, as reported recently in trout [112]. Studies on the functionality of the urea cycle in the early developmental stages in these air-breathing teleosts will be necessary to clear this point, and will also further examine the hypothesis that urea cycle genes were conserved in teleosts during evolution of vertebrates in contrast to the 'gene deletion' hypothesis proposed by Brown and Cohen [10].

1.3. Ammonia tolerance versus toxicity, and its effects on ureogenesis

Ammonia can occur in natural waters as well as in tissue fluids both in unionized (NH_3) and ionized (NH_4^+) forms. The toxicity of ammonia is attributed primarily to the unionized form [94]. Laboratory studies of ammonia toxicity to aquatic animals are extensive in the literature, and many have shown that several factors may change the toxicant effects. These include the pH of the solution, temperature, ionic strength, dissolved oxygen and carbon dioxide [1,2,44,94]. Ammonia can cause reduction in growth rate [53], gill hyperplasia, thickening of the gill epithelium, fusion of lamellae, swollen gills and haemorrhaging [11,76,89,91]. Higher levels of ammonia modify the properties of the blood–brain barrier [21,88], interfere with amino acid transport [56], disrupt cerebral blood flow [6], and interfere with excitatory amino acid neurotransmitter metabolism, particularly that of glutamate and aspartate [45]. NH_4^+ interrupts the ion-exchange mechanism of nerve impulse conduction by directly substituting K^+ [9,21]. Furthermore, ammonia has been shown to change carbohydrate and fat metabolism and ATP levels, not only in the brain, but in other tissues as well [102]. All these toxic effect of ammonia (both NH_3 and NH_4^+) may lead to convulsion, coma and eventually death.

Ammonia toxicity in various ammoniotelic teleosts has been studied extensively and the 96 h LC_{50} value for unionized ammonia was found to be well below 0.1 mmol l^{-1} [12,24,42,95,96]. The 48 h LC_{50} value of total ammonia (TA) for *Cyprinus carpio* was 0.28 mmol l^{-1} [24], and the 24 h LC_{50} value for TA was 0.15 mmol l^{-1} for the trout, *Salmo gairdneri* [68], whereas for the ureotelic alkaline lake Magadi Tilapia, *Oreochromis alcalicus grahami* the 24 h LC_{50} value for TA was 0.75 mmol l^{-1} [100]. However, in the ammoniotelic, but potentially ureogenic singhi catfish (*H. fossilis*), the ambient TA tolerance limit was found to be many fold higher than that in any of these ureogenic and non-ureogenic teleosts [80,81]. To date, this catfish appears to have the maximum capacity of tolerating the external TA (up to 75 mM ammonium chloride) for weeks without mortality [81]. When these fish were exposed to still higher concentrations of TA (100 and 200 mM ammonium chloride), they died within 2–4 days. The decrease in blood pH due to accumulation of acidic metabolites probably from glycolysis and Krebs cycle at high ambient ammonia may not be the cause of death as suggested by Sausa and Meade [85] in Coho salmon, since Hillaby and Randall [44] did not observe any decrease in the blood pH when the TA load in the blood of rainbow trout (*Salmo gairdneri*) was elevated to a toxic level. The pH of the ambient medium decreased only from 6.82 to 6.48 with an increasing concentration of ammonium chloride from 75 to 200 mM [81]. The death could be due to other effects of ammonia accumulated to a toxic level as mentioned above. Large concentrations of ammonia accumulate in all non-cerebral tissues such as liver, kidney, muscle and also in plasma in singhi catfish except in brain when exposed to higher ambient ammonia [81]. High levels of activity of GS [17] and glutamate dehydrogenase (GDH, reductive amination) [26] reported in the brain of singhi catfish and recently in walking catfish, which are also inducible under higher ammonia load (Dutta and Saha, unpublished data), probably help in avoiding accumulation of toxic ammonia in the brain per se by assimilating ammonia to glutamine and glutamate, respectively. This route to detoxify brain ammonia has also been shown to occur in a number of fish species [7,49,54].

One of the major reasons for tolerating such high ambient ammonia by these air-breathing fish could be the presence of a very active urea cycle both in the liver and kidney [78,79], together with the capacity to stimulate ureogenesis under hyperammonia stress [77,80,81]. This facultatively ureogenic air-breathing singhi catfish (*H. fossilis*) also shows a rapid transition from ammoniotelism to ureotelism when exposed to high ambient ammonia to avoid accumulation of ammonia to a toxic level [81]. On exposure to high ambient ammonia, there is an initial accumulation of ammonia in different tissues and plasma accompanied by a significant increase of activity of all the urea cycle enzymes (except arginase) by about 1.5–2.5-fold, resulting in increased synthesis, accumulation and excretion of urea [77,80,81] (see Figs. 2 and 4). It was hypothesized that higher accumulation of tissue ammonia probably serves as a

modulator to induce ureogenesis in these air-breathing fish. This hypothesis was supported further by the results of perfusion experiments performed recently [83], where the liver was loaded with ammonia by infusing ammonium chloride at different rates. As expected, this treatment also led to increased activity of all the urea cycle enzymes (except arginase), and stimulation of the urea excretion rate within 60 min of infusion of ammonium chloride.

In addition to ammonia, certain hormones such as glucocorticoids, glucagon and thyroxine also may play some role in the regulation of ureogenesis in this unique group of air-breathing teleosts as reported in mammals (for review, see Ref. [93]) and in the amphibian, *Xenopus laevis* [8]. Little information is available about the hormonal regulation of ureogenesis in fish. Mommsen et al. [61] studied the effects of several hormones, including glucagon, glucagon-like peptide, epinephrine and vasoactive peptides, on the regulation of urea synthesis in isolated hepatocytes of gulf toadfish, *O. beta*, but could not find any response to any of the hormones. However, GS, which appears to be an important enzyme related to urea synthesis in fish for supplying ammonia nitrogen to CPS III, was found to be stimulated by cortisol released at high levels during confinement/crowding in the same toadfish [47]. Mommsen et al. [62] suggested that a cortisol related stress response may be only one of several mechanisms by which ureogenesis is activated in gulf toadfish rather than higher ammonia load. In Indian air-breathing teleost species, however, higher ammonia load appears to be an important modulator for regulation of ureoge-

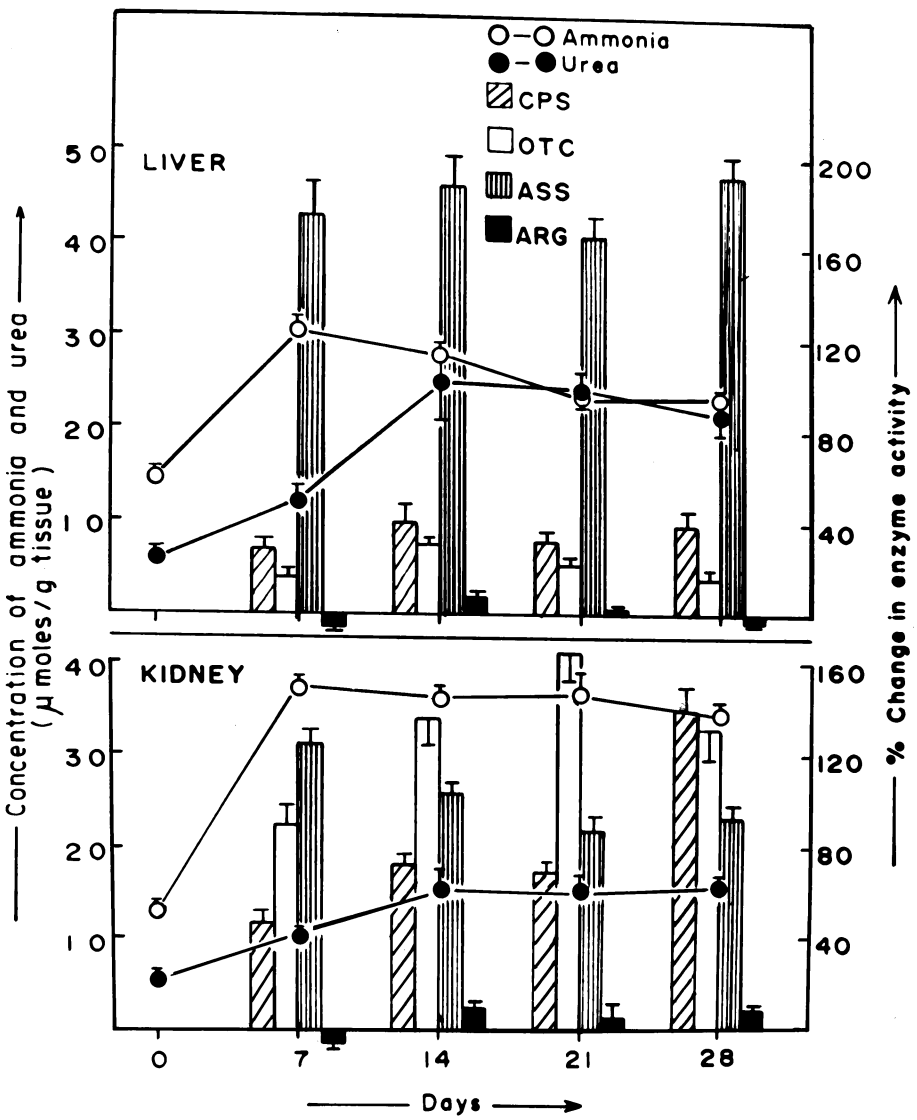

Fig. 2. Alterations in the percentage change of activity (U g^{-1} wet wt) of the urea cycle enzymes with relation to the concentration (μmol g^{-1} wet wt) of ammonia and urea in liver and kidney of *H. fossilis* exposed to 50 mM ammonium chloride. Data taken from Saha and Ratha (1994) [81]. For abbreviations, see Fig. 1; except ASS, argininosuccinate synthetase system, where both argininosuccinate synthetase and argininosuccinate lyase were measured together.

nesis, since the stimulation of ureogenesis along with the increased activities of urea cycle enzymes were found in the perfused liver infused with different concentrations of ammonium chloride in singhi catfish [83]. However, elucidation of the role of hormones in ureogenesis under different stresses in air-breathing teleosts are probably necessary to draw any definite conclusion. Further, there could be other possible mechanism(s) of controlling ureogenesis in air-breathing teleosts: (i) the more abundance of *N*-acetyl-L-glutamate (allosteric regulator) under hyperammonia stress, which can regulate at least the activity of CPS I and III, as shown in rat liver [55]; or (ii) regulation by the limited supply of substrate (ammonia and/or glutamine) as suggested in alkaline lake Magadi tilapia (*O. a. grahami*) [100]. Since, both CPS I-like and CPS III activities are present in these air-breathing catfish [84], CPS I may become saturated first with ammonia, and then CPS III, which may limit the urea synthesis or vice versa. Another possible mechanism could be: (iii) the modification of pre-existing enzymes by higher ammonia load under hyper-ammonia stress. However, the possibility of regulation of urea synthesis at the transcriptional level cannot be ruled out.

Other detoxification pathways such as the conversion of accumulated ammonia to various non-essential free amino acids (FAA) as reported in the mudskipper, *Periophthalmus cantonensis* [49], are probably necessary as additional adaptations in this group of air-breathing fish to tolerate such a high ambient ammonia. As such high levels of activity of GDH and GS have been reported in different tissues of singhi and walking catfish ([17,26]; Dutta and Saha, unpublished data). Studies of alterations of levels of different non-essential FAA along with alterations of various related enzymes under hyper-ammonia stress would be necessary to clarify this point.

1.4. Exposure to air and ureogenesis

Since excretion of ammonia per se is extremely difficult for most animals living outside water, the changes in the pattern of end-product nitrogen metabolism or excretion is absolutely necessary for any aquatic animal to migrate from water to land, or for living temporarily outside water for various periods [35]. However, as an exception, some terrestrial snails [92], crabs [28,40] and isopods [114] excrete significant portions of their nitrogenous wastes by ammonia volatilization. Various adaptations related to nitrogen metabolism have been reported in amphibious fish during their life outside water or in semidry condition, such as mudskippers [35,37–39], and a marine teleost, *Blennius pholis* [27].

The Indian air-breathing teleost species are also amphibious according to the definition of Gordon et al.

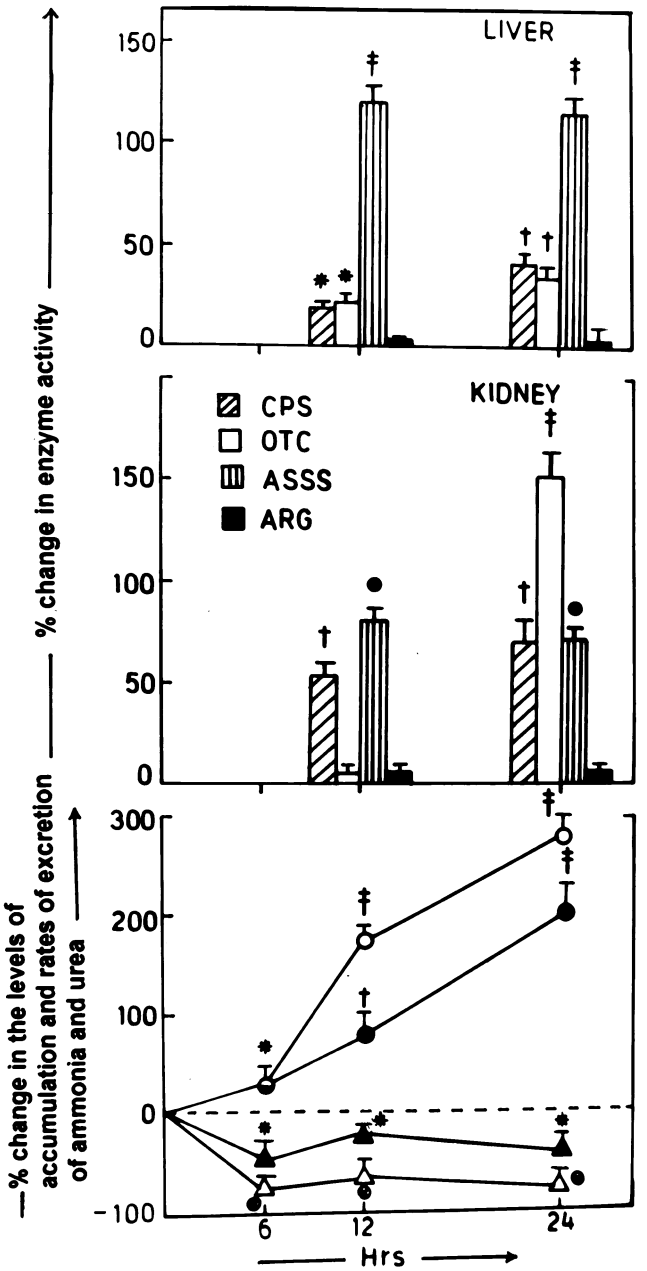

Fig. 3. Percentage change in the levels of ammonia (○) and urea (●), rates of excretion of ammonia (△) and urea (▲) and the urea cycle enzymes activity in *H. fossilis* during different hours of aerial exposure. Data taken from Ratha et al. (1995) [74]. *, ●, †, ‡: *P*-values significant at <0.05, <0.01, <0.005 and <0.001 level, respectively. For abbreviations, see Fig. 1; except ASSS, argininosuccinate synthetase system.

[37]: "amphibious fishes are those which spend periods of time out of water, on or above the ground surface as normal parts of their life histories". Many species of the Indian air-breathing teleosts are known to live for months in a semidry condition inside mud in response to habitat drying [87] and are also able to survive totally outside water for hours ranging from 10 to 100 h [79]. Therefore, various adaptations to nitrogen metabolism are also anticipated in this group of Indian

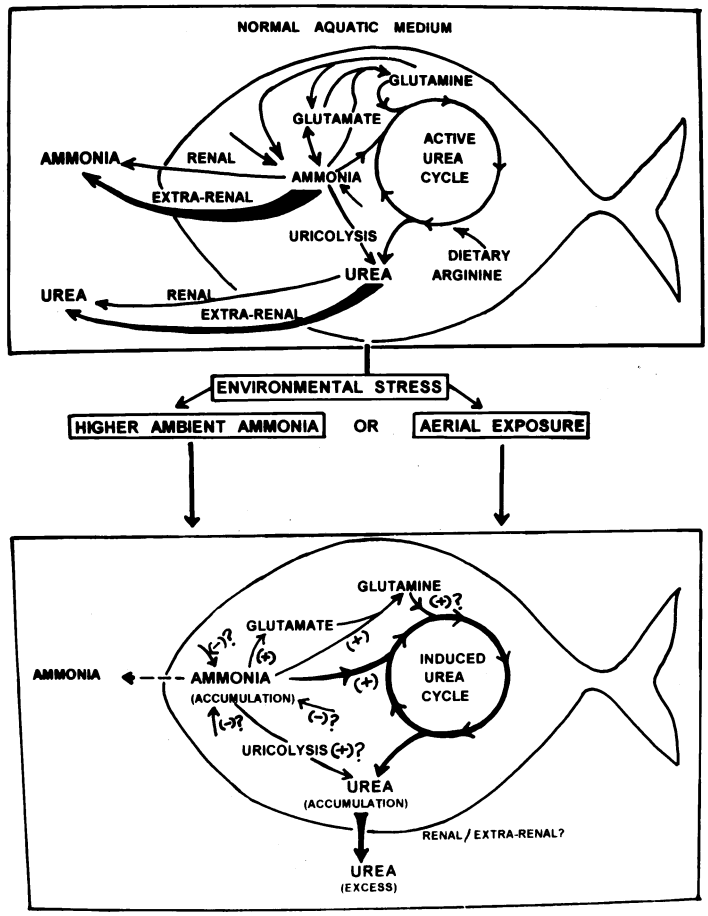

Fig. 4. A model explaining the regulation of ureogenesis in air-breathing teleosts during hyper-ammonia stress either due to exposure to higher ambient ammonia or due to aerial exposure. +, stimulation; −, inhibition; ?, not confirmed; ---, low level; —, moderate level; ▬, high level. Under normal aquatic conditions fish primarily excrete ammonia mostly through the extra-renal sources. A significant amount of urea is also excreted mostly synthesized via the active urea cycle. During exposure to higher ambient ammonia there is an uptake of ammonia from the external environment leading to the higher accumulation of ammonia per se, and also during aerial exposure due to tremendous decrease of ammonia excretion. The higher accumulation of ammonia in the body in both conditions probably acts as a modulator to stimulate the activity of the urea cycle enzymes leading to enhanced synthesis of urea, accumulation, and excretion, thereby turning the fish from ammoniotelism to ureotelism.

air-breathing fish especially during their amphibious life. Ramaswamy and Reddy [70] demonstrated a marked shift towards ureotelism from ammoniotelism in two Indian obligatory air-breathing teleosts (*Anabas scandens* and *Channa gachua*) but not in the facultative air-breathing teleost, *Mystus vittalus* when exposed to air for 5 or 10 h. A similar transition to ureotelism from ammoniotelism has been reported in singhi catfish (*H. fossilis*), when exposed to air for 24 h [74] (Fig. 3). A gradual increase of ammonia accumulation in situ (from 5.47 ± 0.57 to 20.73 ± 2.28 μmol g^{-1} body wt after 24 h of aerial exposure) during aerial exposure probably due to severe decrease of ammonia excretion rate might have resulted in the stimulation of activity of all the urea cycle enzymes, (except arginase), resulting to more synthesis of urea from accumulated ammonia, and its accumulation in the body to higher levels (from 4.09 ± 0.64 to 12.4 ± 1.1 μmol g^{-1} body wt after 24 h of aerial exposure) ([74]; see also, Figs. 3 and 4). The tissue water content did not change much in these fish even after 24 h of aerial exposure [74]. In another set of experiments, a set of singhi catfish was kept inside mud mimicking their habitat in response to habitat drying outside the laboratory. The following observations were made with relation to ureogenesis after removal of fish from mud: urea accumulated to a very high level in plasma and in all tissues, whereas ammonia did not accumulate much in any of the tissues. The activities of all the urea cycle enzymes (except arginase) (both in the liver and kidney tissues) were induced by 3–4-fold compared to control fish kept in water. When these fish were re-immersed in water, there was an initial increase of urea excretion rate of about 10–15-fold within 6 h followed by an increase of ammonia excretion rate (Saha, Das, Dutta and Goswami, unpublished results). The results of this experiment clearly indicated that a transition from ammoniotelism to ureotelism occurs in singhi catfish remaining inside mud during habitat drying as a physiological

adaptation to conserve water and to avoid accumulation of ammonia per se to a toxic level. However, it is difficult to say with the available data whether the formation of ammonia decreased in fish living in semidry condition. Similar observations were also made in African lungfish (*Protopterus aethiopicus*) living inside mud cocoons during aestivation [51]. However, in contrast to singhi catfish, no induction of activities of urea cycle enzymes was noted in lungfish. The hypothesis has been put forward explaining the regulation of ureogenesis during exposure to higher ambient ammonia in this group of air-breathing fish, (i.e. a higher accumulation of ammonia, which results from reduced excretion due to lack of water, acts a modulator for stimulation of ureogenesis) probably also holds good during exposure to air and also while living in semidry condition (Fig. 4). However, with the available data the real mechanism(s) of stimulation of ureogenesis in these air-breathing fish under hyperammonia stress is not clear and should be studied in detail at the molecular level including the purification and characterization of all the urea cycle enzymes, and the abundance of mRNA level of individual enzymes under hyperammonia stress. The role of other detoxification pathways, such as the synthesis of various non-essential amino acids from ammonia by GDH, GS and various transaminase enzymes as reported in the mudskipper, *P. cantonensis* [50], which may serve as an additional adaptation to live outside water for varying periods, or while living inside mud during habitat drying needs to be investigated in these air-breathing teleost species.

Acknowledgements

The authors thank the North-Eastern Hill University, Shillong for providing the laboratory facilities and for partial financial assistance. These works were partly supported by the University Grants Commission under the Department of Research Studies project and the Council of Scientific and Industrial Research, New Delhi. The authors wish to thank sincerely Dr T.P. Mommsen for his valuable comments and also for critically reviewing the earlier draft of this manuscript.

References

[1] Alabaster JS, Herbert DWH. Influence of carbondioxide on the toxicity of ammonia. Nature 1954;174:404.
[2] Alabaster JS, Shurben DG, Knowles G. The effect of dissolved oxygen and salinity on the toxicity of ammonia smolts of salmon *Salmo salar* L. J Fish Biol 1979;15:705–12.
[3] Anderson PM. Urea cycle in fish: molecular and mitochondrial studies. In: Hoar WS, Randall DJ, Farrell AP, series editors. Fish Physiology, Vol. 14. In: Wood CM, Shuttleworth TJ, editors. Ionoregulation: Cellular and Molecular Approaches. New York: Academic Press, 1995:57–83.
[4] Anderson PM. Molecular aspects of carbamoyl phosphate synthesis. In: Evans DH, series editor. Animal Physiology: Evolutionary and Ecological Perspectives. In: Walsh PJ, Wright PA, editors. Nitrogen Metabolism and Exretion. Boca Raton: CRC Press, 1995:33–50.
[5] Anderson PM, Walsh PJ. Subcellular localization and biochemical properties of the enzymes of carbamoyl phosphate and urea synthesis in the batrachoidid fishes *Opsanus beta*, *Opsanus tau* and *Porichthys notatus*. Biol J Exp 1995;198:755–66.
[6] Andersson KE, Brandt L, Hindfelt B, Ljunggren B. Cerebrovascular effects of ammonia in vitro. Acta Physiol Scand 1981;113:349–53.
[7] Arillo A, Margiocco C, Medlodia F, Mensi P, Schenone G. Ammonia toxicity mechanism in fish: Studies on rainbow trout (*Salmo gairdneri* Rich). Ecotoxicol Env Safety 1981;5:316–28.
[8] Balinisky JB, Teresa LC, Mattheyse FJ. The effect of thyroxine and hypertonic environment on the enzymes of the urea cycle in *Xenopus laevis*. Comp Biochem Physiol 1972;43B:83–95.
[9] Binstock L, Lecar H. Ammonium ion currents in the squid giant axon. J Gen Physiol 1969;53:342–61.
[10] Brown GWJr, Cohen PP. Comparative biochemistry of urea synthesis-III. Activities of urea cycle enzymes in various higher and lower vertebrates. Biochem J 1960;75:82–91.
[11] Burrows RE. Effects of accumulated excretory products on hatchery-reared salmonids. Fish Wildl Serv Bureau Sport Fish Wildl. US Res Rept 1964;66:1–12.
[12] Campbell JW. Excretory nitrogen metabolism. In: Prosser CL, editor. Environmental and Metabolic Animal Physiology. New York: Wiley-Liss, 1991:277–324.
[13] Campbell JW, Anderson PM. Evolution of mitochondrial enzyme system in fish: the mitochondrial synthesis of glutamine and citrulline. In: Hochachka PW, Mommsen TP Jr., editors. Biochemistry and Molecular Biology of Fishes, vol. 1. Amsterdam: Elsevier, 1991:43–76.
[14] Campbell JW, Smith DD Jr., Vorhaben JE. Avian and mammalian mitochondrial ammonia-detoxifying systems in tortoise liver. Science 1985;228:349–51.
[15] Cao X, Kemp JR, Anderson PM. Subcellular localization of two glutamine-dependent carbamoyl phosphate synthetase and related enzymes in liver of *Micropterus salmoides* (largemouth bass) and properties of isolated liver mitochondria: comparative relationships with elasmobranchs. J Exp Zool 1991;258:24–33.
[16] Casey CA, Anderson PM. Subcellular location of glutamine synthetase and urea cycle enzymes in liver of spiny dogfish (*Squalus acanthias*). J Biol Chem 1982;257:8449–53.
[17] Chakravorty J, Saha N, Ratha BK. A unique pattern of tissue distribution and sub-cellular localization of glutamine synthetase in a freshwater air-breathing teleost, *Heteropneustes fossilis*. Biochem Int 1989;19:519–27.
[18] Chatrath KJS. Wetlands of India. New Delhi: Asish, 1992.
[19] Chiu YN, Austic RE, Rumsey GL. Urea cycle activity and arginine formation in rainbow trout (*Salmo gairdneri*). J Nutr 1986;116:1640–50.
[20] Cohen PP. Evolutionary and comparative aspects of urea biosynthesis. In: Grisolia S, Baguena R, Mayor F, editors. The Urea Cycle. New York: Wiley, 1976:21–8.
[21] Cooper AJL, Plum F. Biochemistry and physiology of brain ammonia. Physiol Rev 1987;67:440–519.
[22] Cvancara VA. Studies on tissues arginase and ureogenesis in freshwater teleosts. Comp Biochem Physiol 1969;30:489–96.
[23] Cvancara VA. Comparative study of liver uricase in freshwater teleosts. Comp Biochem Physiol 1969;28:725–32.

[24] Dabrowska H, Wlasow T. Sublethal effect of ammonia on certain biochemical and hematological indicators in common carp. Comp Biochem Physiol 1986;83C:179–84.
[25] Danulat E, Kempe S. Nitrogenous waste excretion and accumulation of urea and ammonia in *Chalcalburnus tarichi* (Cyprinidae) endemic to the extremely alkaline Lake Van (Eastern Turkey). Fish Physiol Biochem 1992;9:377–86.
[26] Das JR, Saha N, Ratha BK. Tissue distribution and subcellular localization of glutamate dehydrogenase in a freshwater air-breathing teleost *Heteropneustes fossilis*. Biochem Syst Ecol 1991;19:207–12.
[27] Davenport J, Sayer MD. Ammonia and urea excretion in the amphibious teleost *Blennius pholis* (L.) in seawater and in air. Comp Biochem Physiol 1986;84A:189–94.
[28] DeVries MC, Wolcott DL. Gaseous ammonia evolution is coupled to reprocessing of urine at the gills of ghost crabs. J Exp Zool 1993;267:97–103.
[29] Dkhar J, Saha N, Ratha BK. Ureogenesis in a freshwater teleost: an unusual sub-cellular localization of ornithine-urea cycle enzymes in the freshwater air-breathing teleost, *Heteropneustes fossilis*. Biochem Int 1991;25:1061–9.
[30] Dutta HM, Munshi JSD. Functional morphology of air-breathing fishes. A review. Proc Indian Acad Sci (Animal Sci) 1985;94:359–75.
[31] Goldstein L, Forster RP. The role of uricolysis in the production of urea by fishes and other aquatic vertebrates. Comp Biochem Physiol 1965;14:567–76.
[32] Goldstein L, Forster RP. Urea biosynthesis and excretion in freshwater and marine elasmobranchs. Comp Biochem Physiol 1971;39B:415–21.
[33] Goldstein L, Harley-Dewitt S, Forster RP. Activities of ornithine-urea cycle enzymes and of trimethylamine oxidase in the coelacanth *Latimeria chalumnae*. Comp Biochem Physiol 1973;44B:357–62.
[34] Goldstein L, Janssens PA, Forster RP. Lungfish *Neoceratodus forsteri*; activities of ornithine-urea cycle and enzymes. Science 1967;157:316–7.
[35] Gordon MS. Patterns of nitrogen excretion in amphibious fishes. In: Schmidt-Nielsen B, Kerr DWS, editors. Urea and the Kidney. Amsterdam: Excerpta Medica, 1970:238–42.
[36] Gordon MS, Boetius I, Boetius I, Evans DH, Mc Carthy R, Oglesby LC. Salinity adaptation in the mudskipper fish, *Periophthalmus sobrinus*. Hvalradets Skrifter Norske Videnskap Akad. Oslo 1965;48:85–95.
[37] Gordon MS, Boetius I, Evans DH, McCarthy R, Oglesby LC. Aspects of the physiology of terrestrial life in amphibious fishes. I. The mudskipper, *Periophthalmus sobrinus*. J Exp Biol 1969;50:141–9.
[38] Gordon MS, Fisher S, Tarifeno E. Aspects of physiology of terrestrial life in amphibious fishes. II. The Chilean clingfish, *Sicyases sanguineus*. J Exp Biol 1970;53:559–72.
[39] Gordon MS, Ng Ww-s, Yip Ay-W. Aspects of the physiology of terrestrial life in amphibious fishes. III. The Chines mudskipper *Periophthalmus cantonensis*. J Exp Biol 1978;72:57–75.
[40] Greenaway P, Nakamura T. Nitrogenous excretion in two terrestrial crabs (*Gecarcoidea natalis* and *Geograpsus gray*). Physiol Zool 1991;64:767–86.
[41] Hayashi S, Fujiwara S, Naguchi T. Degradation of uric acid in fish liver peroxisomes. Intraperoxisomal localization of hepatic allantoicase and purification of its peroxisomal membrane-bound form. J Biol Chem 1989;264:3211–5.
[42] Haywood GP. Ammonia toxicity in teleost fishes: a review. Can Tech Rep Fish Aquat Sci 1983;1177:35.
[43] Helbing CC, Atkinson BG. 3,5,3′-Triiodo-thyronine-induced carbamyl phosphate synthetase gene expression is stabilized in the liver of *Rana catesbeiana* tadpoles during heat shock. J Biol Chem 1994;269:11743–50.
[44] Hillaby BA, Randall DJ. Acute ammonia toxicity and ammonia excretion in rainbow trout (*Salmo gairdneri*). J Fish Res Bd Can 1979;36:621–9.
[45] Hindfelt B, Plum F, Duffy TE. Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. J Clin Invest 1977;59:386–96.
[46] Hong J, Salo WL, Lusty CJ, Anderson PM. Carbamyl phosphate synthetase III, an evolutionary intermediate in the transition between glutamine-dependent and ammonia-dependent carbamyl phosphate synthetases. J Mol Biol 1994;243:131–40.
[47] Hopkins TE, Wood CM, Walsh PJ. Interactions of cortisol and nitrogen metabolism in the ureogenic gulf toadfish *Opsanus beta*. J Exp Biol 1995;198:2229–35.
[48] Huggins AK, Skutsch G, Baldwin E. Ornithine-urea cycle enzymes in teleostean fish. Comp Biochem Physiol 1969;28:587–602.
[49] Iwata K. Nitrogen metabolism in the mudskipper, *Periophthalmus cantonensis*: changes in free amino acids and related compounds in various tissues under conditions of ammonia loading, with reference to its high ammonia tolerance. Comp Biochem Physiol 1988;91A:499–508.
[50] Iwata K, Kakuta I, Ikeda M, Kimoto S, Wada N. Nitrogen metabolism in mudskipper, *Periophthalmus cantonensis*: a role of free amino acids in detoxification of ammonia produced during its terrestrial life. Comp Biochem Physiol 1981;68A:589–96.
[51] Janssens PA, Cohen PP. Biosynthesis of urea in the aestivating African lungfish and in *Xenopus laevis* under conditions of water shortage. Comp Biochem Physiol 1968;24:887–98.
[52] Johansen K. Air breathing in fishes. In: Hoar WS, Randall DJ, editors. Fish Physiology, Vol IV. New York: Academic Press, 1970:361–411.
[53] Larmoyeux JD, Piper RG. Effects of water rinse on rainbow trout in hatcheries. Prog Fish Cult 1973;35:2–8.
[54] Levi G, Morisi G, Coletti A, Catanzaro R. Free amino acids in fish brain: normal levels and changes upon exposure to high ammonia concentrations in vivo, and upon incubation of brain slices. Comp Biochem Physiol 1974;49A:623–36.
[55] Lund P, Wiggins D. Is *N*-acetylglutamate a short-term regulator of urea synthesis? Biochem J 1984;218:991–4.
[56] Mans AM, Biebuyck JF, Hawkins RA. Ammonia selectively stimulates neutral amino acid transport across blood–brain barrier. Am J Physiol 1983;245:C74–7.
[57] McCarthy JJ, Whitedge TE. Nitrogen excretion by anchovy, (*Engraulis mordax*) and jack mackerel (*Trachurus symmetricus*). Fish Bull 1972;70:395–401.
[58] McGeer J, Wright PA, Wood CM, Wilkie MP, Majur CF, Iwama GK. Nitrogen excretion in four species of fish from an alkaline lake. Trans Am Fish Soc 1994;123:824–9.
[59] McKenzie DJ, Randall DJ. Does *Amia calva* aestivate? Fish Physiol Biochem 1990;8:147–54.
[60] Meijer AJ, Lamers WH, Chamuleau RAFM. Nitrogen metabolism and ornithine cycle function. Physiol Rev 1990;70:701–48.
[61] Mommsen TP, Danulat E, Walsh PJ. Hormonal regulation of metabolism in hepatocytes of the ureogenic teleost *Opsanus beta*. Fish Physiol Biochem 1991;9:247–52.
[62] Mommsen TP, Walsh PJ. Evolution of urea synthesis in vertebrates: the piscine connection. Science 1989;243:72–5.
[63] Mommsen TP, Walsh PJ. Urea synthesis in fishes: evolutionary and biochemical perspectives. In: Hochachka PW, Mommsen TP, editors. Biochemistry and Molecular Biology of Fishes, Vol. 1. Amsterdam: Elsevier, 1991:137–63.
[64] Mommsen TP, Walsh PJ. Biochemical and environmental perspectives on nitrogen metabolism. Experientia 1992;48:583–93.
[65] Morii H, Nishikata K, Tamura O. Nitrogen excretion of mudskipper fish *Periophthalmus pectinirostris* in water and on land. Comp Biochem Physiol 1978;60A:189–93.

[66] Munshi JSD, Ghosh TK. Metabolic wheel hypothesis as applied to air-breathing fishes of India. In: Singh HR, editor. Advances in Fish Biology, vol. 1. Delhi: Hindustan, 1994:70–8.
[67] Munshi JSD, Hughes GM. Air-breathing Fishes: Their Structure and Life History. New Delhi: Oxford Publication and IBH, 1992.
[68] Olson KR, Fromm PO. Excretion of urea by two teleosts exposed to different concentrations of ambient ammonia. Comp Biochem Physiol 1971;40A:999–1007.
[69] Perlman DF, Goldstein L. Nitrogen metabolism. In: Shuttleworth TJ, editor. Physiology of Elasmobranch Fishes. Heidelberg: Springer-Verlag, 1988:253–76.
[70] Ramaswamy M, Reddy TG. Ammonia and urea excretion in three species of air-breathing fish subjected to aerial exposure. Proc Indian Acad Sci (Animal Sci) 1983;92:293–7.
[71] Randall DJ, Burggren WW, Farrel AP, Haswell MS. The Evolution of Air-Breathing in Vertebrates. Cambridge, 113 p: Cambridge University Press, 1981.
[72] Randall DJ, Wright PA. Ammonia distribution and excretion in fish. Fish Physiol Biochem 1987;3:107–20.
[73] Randall DJ, Wood CM, Perry SF, Bergman H, Maloiy GMO, Mommsen TP, Wright PA. Urea excretion as a strategy for survival in very alkaline environment. Nature 1989;337:165–6.
[74] Ratha BK, Saha N, Rana RK, Chaudhury B. Evolutionary significance of metabolic detoxification of ammonia to urea in an ammoniotelic freshwater teleost, *Heteropneustes fossilis* during temporary water deprivation. Evolucion Biol 1995;8, 9:107–17.
[75] Read LJ. The presence of high ornithine-urea cycle enzymes activity in the teleost *Opsanus tau*. Comp Biochem Physiol 1971;39B:409–13.
[76] Reichenback-Klinke HH. Untersuchungen uber die Einwirkung des Fischorganismus. Arch Fischereiwiss 1967;17:122–32.
[77] Saha N, Ratha BK. Effect of ammonia stress on ureogenesis in a freshwater air-breathing teleost, *Heteropneustes fossilis*. In: Kon OL, Chung MC, Hwang PLH, Leong S, Loke KH, Thiyagaraja P, Wong PT, editors. Contemporary Themes in Biochemistry, vol. 6. Cambridge: Cambridge University Press, 1986:342–3.
[78] Saha N, Ratha BK. Active ureogenesis in a freshwater air-breathing teleost, *Heteropneustes fossilis*. J Exp Zool 1987;241:137–41.
[79] Saha N, Ratha BK. A comparative study of ureogenesis in freshwater air-breathing teleosts. J Exp Zool 1989;252:1–8.
[80] Saha N, Ratha BK. Alterations in the excretion pattern of ammonia and urea in a freshwater air-breathing teleost, *Heteropneustes fossilis* (Bloch) during hyper-ammonia stress. Indian J Exp Biol 1990;28:597–9.
[81] Saha N, Ratha BK. Induction of ornithine-urea cycle in a freshwater teleost, *Heteropneustes fossilis*, exposed to high concentrations of ammonium chloride. Comp Biochem Physiol 1994;108B:315–25.
[82] Saha N, Chakravorty J, Ratha BK. Diurnal variation in renal and extra-renal excretion of ammonia-N and urea-N in a freshwater air-breathing teleost, *Heteropneustes fossilis* (Bloch). Proc Indian Acad Sci (Animal Sci) 1988;97:529–37.
[83] Saha N, Dkhar J, Ratha BK. Induction of ureogenesis in perfused liver of a freshwater teleost, *Heteropneustes fossilis*, infused with different concentrations of ammonium chloride. Comp Biochem Physiol 1995;112B:733–41.
[84] Saha N, Dkhar J, Anderson PM, Ratha BK. Carbamyl phosphate synthetases in an air-breathing teleost, *Heteropneustes fossilis*. Comp Biochem Physiol 1997;116B:57–63.
[85] Sausa RJ, Meade TL. The influence of ammonia on the oxygen delivery system of coho salmon haemoglobin. Comp Biochem Physiol 1977;58A:23–58.
[86] Sayer MDJ, Davenport J. The relative importance of the gills to ammonia and urea excretion in five seawater teleost species. J Fish Biol 1987;31:561–7.
[87] Sayer MDJ, Davenport J. Amphibious fish: why do they leave water? Rev Fish Biol Fisheries 1991;1:159–81.
[88] Sears ES, McCandless DW, Chandler MD. Disruption of the blood-brain barrier in hyperammonemic coma and the pharmacologic effects of dexamethasone and difluoromethyl ornithine. J Neurosci Res 1985;14:255–61.
[89] Smart G. The effect of ammonia exposure on gill structure of the rainbow trout. J Fish Biol 1976;8:471–9.
[90] Smith HW. The excretion of ammonia and urea by the gill of fish. J Biol Chem 1929;82:727–42.
[91] Smith CE, Piper RG. Lesions associated with chronic exposure to ammonia. In: Ribelin WE, Migaki G, editors. The Pathology of Fishes. Madison: University of Wisconsin Press, 1975:497–517.
[92] Speeg KV, Campbell JW. Formation and volatilization of ammonia gas by terrestrial snails. Am J Physiol 1968;214:1392–402.
[93] Takiguchi M, Mori M. Transcriptional regulation of genes of ornithine cycle enzymes. Biochem J 1995;312:649–59.
[94] Thurston RV, Russo RC, Vinogradov GA. Ammonia toxicity to fishes. The effect of pH on the toxicity of the un-ionized ammonia species. Environ Sci Tech 1981;15:837–40.
[95] Thurston RV, Russo RC, Phillips GR. Acute toxicity of ammonia to fathead minnows. Trans Am Fish Soc 1983;112:705–11.
[96] Thurston RV, Russo RC, Vinogradov GA. Acute toxicity of ammonia to rainbow trout. Trans Am Fish Soc 1983;112:696–704.
[97] Vorhaben JE, Campbell JW. Glutamine synthetase. A mitochondrial enzyme in uricotelic species. J Biol Chem 1972;247:2763–7.
[98] Vorhaben JE, Campbell JW. Submitochondrial localization and function of enzymes of glutamine metabolism in avian liver. J Cell Biol 1977;73:300–10.
[99] Walton MJ, Cowey CB. Aspects of intermediary metabolism in salmonid fish. Comp Biochem Physiol 1982;73:59–79.
[100] Walsh PJ, Bergman HL, Narahara A, Wood CM, Wright PA, Randall DJ, Maira JN, Laurent P. Effect of ammonia on survival, swimming, and activities of enzymes of nitrogen metabolism in the Lake Magadi tilapia *Oreochromis alcalicus grahami*. J Exp Biol 1993;180:323–7.
[101] Walsh PJ, Danulat E, Mommsen TP. Variation in urea excretion in the Gulf toadfish *Opsanus beta*. Mar Biol 1990;106:323–8.
[102] Wiechetek M, Breves G, Holler H. The effect of ammonia ion concentration on the activity of adenylate cyclase in various rat tissues in vitro. J Exp Physiol 1979;64:169–74.
[103] Wilkie MP, Wood CM. Nitrogenous waste excretion, acid-base regulation, and ionoregulation in rainbow trout (*Oncorhynchus mykiss*) exposed to extremely alkaline water. Physiol Zool 1991;64:1069–86.
[104] Wilkie MP, Wright PA, Iwama GK, Wood CM. The physiological responses of the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), a resident of highly alkaline Pyramid Lake (pH 9.4), to challenge at pH 10. J Exp Biol 1993;175:173–94.
[105] Wilkie MP, Wright PA, Iwama GK, Wood CM. The physiological adaptations of the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) following transfer from well water to the highly alkaline waters of Pyramid Lake, Nevada (pH 9.4). Physiol Zool 1994;67:355–80.

[106] Wilson RP. Nitrogen metabolism in channel catfish, *Ictalurus punctatus*. II. Evidence for an apparent incomplete ornithine-urea cycle. Comp Biochem Physiol 1973;46B:625–34.
[107] Wood JD. Nitrogen excretion in some marine teleosts. Can J Biochem Physiol 1958;36:1237–42.
[108] Wood CM. Ammonia and urea metabolism and excretion. In: Evans DH, editor. The Physiology of Fishes. London: CRC Press, 1993:379–425.
[109] Wood CM, Perry SF, Wright PA, Bergman HL, Randall DJ. Ammonia and urea dynamics in the Lake Magadi tilapia, a ureotelic teleost fish adapted to an extremely alkaline environment. Resp Physiol 1989;77:1–20.
[110] Wright PA. Nitrogen excretion and enzyme pathways for ureagenesis in freshwater tilapia (*Oreochromis nilotica*). Physiol Zool 1993;66:881–901.
[111] Wright PA. Nitrogen excretion: Three end products, many physiological roles. J Exp Biol 1995;198:273–81.
[112] Wright PA, Felskie A, Anderson PM. Induction of ornithine-urea cycle enzymes and nitrogen metabolism and excretion in rainbow trout (*Oncorhynchus mykiss*) during early life stages. J Exp Biol 1993;198:127–35.
[113] Wright PA, Iwama GK, Wood CM. Ammonia and urea excretion in Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) adapted to the highly alkaline Pyramid Lake (pH 9.4). J Exp Biol 1995;175:153–72.
[114] Wright JC, O'Donnell MJ. Total ammonia concentration and pH of haemolymph, pleon fluid and maxillary urine in *Porcellio scaber* Lattreille (*Isopoda Oniscidea*): relationships to ambient humidity and water vapour uptake. J Exp Biol 1993;176:233–46.