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Diurnal variation in renal and extra-renal excretion of ammonia-N and urea-N in a freshwater air-breathing teleost, *Heteropneustes fossilis* (Bloch)

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Abstract. Diurnal pattern of excretion of ammonia-N and urea-N through the renal and extra-renal sources were studied in a freshwater air-breathing teleost, *Heteropneustes fossilis* in its aquatic medium. Ammonia-N was the major nitrogen excretory product, however, the rate of excretion of urea-N was comparatively higher than other freshwater teleosts. Almost all ammonia-N (>99%) and about 75% of urea-N were excreted through the extra-renal sources. The excretion rate of urea-N was about 10 times higher than ammonia-N through the renal and ammonia-N was 7–8 times higher than urea-N through extra-renal sources. Existence of a functional ornithine-urea cycle reported in *Heteropneustes fossilis* has been suggested to be the reason for this increased rate of urea excretion.

Nitrogen excretion (total ammonia-N + urea-N) during the night was significantly higher than the day. A higher rate of nitrogen metabolism at night has been suggested for *Heteropneustes fossilis* which has been reported to be nocturnal in habit.

Keywords. Diurnal variation; nitrogen excretion; renal and extra-renal excretion; ammonia-N; urea-N; freshwater air-breathing teleost.

1. Introduction

Freshwater fishes are ammonotelic excreting ammonia as the major nitrogen excretory product primarily through the gills by diffusion (Smith 1929; Forster and Goldstein 1969; Vellas and Serfaty 1974; Walton and Cowey 1982). They are reported to lack a functional ornithine-urea cycle to convert ammonia to urea (Brown and Cohen 1960; Huggins *et al* 1969; Wilson 1973). However, small amounts of urea in different tissues (Holmes and Donaldson 1969) and also as an excretory product (Smith 1929; Brett and Groves 1979; Vellas 1981) have been reported in some freshwater teleosts. It is reported to be produced either from dietary arginine in the presence of arginase (Cvancara 1969a; Saha and Ratha 1987) or through the uricolytic pathway (Goldstein and Forster 1965; Cvancara 1969b; Vellas 1981; Saha and Ratha 1987). Active ureogenesis and ureotelism are characters associated with terrestrial animals where the activity of ornithine-urea cycle enzymes are very high (Cohen 1976). Among freshwater teleosts, there are a few air-breathing species which are capable of surviving temporary water deprivation (Saha 1986) during which ammonia excretion by diffusion through the gills is not possible. Due to their facultative amphibious nature, they are expected to have some special physiological adaptation during their life outside water for detoxification of accumulated ammonia *in vivo*. Saha and Ratha (1987) have reported the

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presence of a complete ornithine-urea cycle with high activity of all the enzymes in the liver and kidney besides a functional uricolytic pathway in one of the freshwater teleosts, *Heteropneustes fossilis*. Having the capacity to convert sufficient amount of ammonia to urea through ornithine-urea cycle, *H. fossilis* should show the nitrogen excretion pattern different from purely aquatic species. This paper presents the findings on the pattern of ammonia-N and urea-N excretion during day and night and through the renal and extra-renal sources in male *H. fossilis*.

2. Materials and methods

2.1 *Animal*

H. fossilis weighing 30–35 g were purchased from commercial sources in late summer and maintained in plastic aquaria in the laboratory at 20 ± 20°C with 12 h : 12 h light and dark period. Minced pig liver was supplied as food and water was changed on alternate days. The fishes were used after 4 weeks of acclimatization to the laboratory conditions when mortality rate was almost zero and food consumption normal. Food was withheld for 24 h prior to and during the experiment. The experiments were carried out at constant temperature and light conditions to which the fishes are acclimatized.

2.2 *Renal and extra-renal excretion*

Renal and extra-renal excretion were studied in male *H. fossilis* as catheterisation could be successful only in male fish. A 6 cm catheter prepared from polyethylene tubings (0·8 mm diameter) was introduced into the urinary bladder of each fish through the ureter and tied along the copulatory bursa. A balloon was tied at the distal end of the catheter for collection of urine. Each catheterised fish was released at 6 AM in a separate jar containing 1000 ml of bacteria free filtered tap water with 10 mg of streptopenicillin (to stop bacterial growth in the medium). All the jars were covered with bilayers of cheese cloth. The urine was collected after 12 h day and 12 h night at 6 PM and next day at 6 AM respectively by puncturing the distal end of the balloon. The volume of urine collected each time was recorded. The amount of ammonia-N and urea-N excreted through renal sources were estimated in the urine and through extra-renal sources (gills, body surface and faeces) were estimated in the water medium collected at the time of urine collection.

2.3 *Estimations*

2.3a *Ammonia-N*: Ammonia-N was estimated colorimetrically following the method of Kawakubo *et al* (1983) by collecting it in 0·1 N HCl after isolating from the sample by alkalisation in a suitably modified diffusion bottle. The concentration of ammonia-N was calculated from a linear standard graph prepared following the same method using different concentrations of NH_4Cl.

2.3b *Urea-N*: Urea-N concentration in the urine and water medium was estimated by converting urea completely to ammonia by urease (Sigma type VI) prepared in 0·1 M phosphate buffer (pH 7·5) and then estimating the amount of ammonia formed following the above method. The concentration of urea-N was calculated from the difference of ammonia-N concentration: [total ammonia-N (fluid ammonia-N + ammonia-N formed from urea-N)—fluid ammonia-N].

2.4 *Chemicals*

Urease was obtained from Sigma Chemical Co., USA and other chemicals used were of analytical grade. Deionized and double glass distilled ammonia free water was used in all preparations.

3. Results

The major nitrogenous excretory product in *H. fossilis* was found to be ammonia (table 1). It accounted for about 85% of the total ammonia-N and urea-N excreted during 24 h. However, during night total N excretion was significantly higher ($P < 0.05$) than the day. The urine output was also significantly higher ($P < 0.001$) at night than the day. However, the concentration of ammonia-N and urea-N did not differ in the urine produced during the day and the night (table 2).

Almost all ammonia-N (> 99%) and most of urea-N (~75%) were excreted through the extra-renal sources (table 3). However, the renal nitrogen excretion was predominantly of urea-N. The ratio of ammonia-N : urea-N excreted through extra-renal sources ranged between 6·4–8·6 and through renal sources 0·11–0·12. Renal excretion of both ammonia-N ($P < 0.02$) and urea-N ($P < 0.05$) and extra-renal excretion of urea-N ($P < 0.001$) were significantly higher at night than day. Total excretion of ammonia-N did not differ significantly between day and night while the excretion of total urea-N was found to be significantly higher ($P < 0.05$) during the night than day. The ammonia-N : urea-N ratio, thus, decreased from 6·6 during the day to 4·7 at night.

Comparison of data on total ammonia-N and urea-N excreted by various fish species (table 4) indicate that ammonia-N : urea-N excretion ratio is higher in purely

Table 1. Diurnal variation of ammonia-N and urea-N excretion (μg N/g body wt/12 h).

Duration	Ammonia-N	Urea-N	Total ammonia-N + urea-N
12 h day (6 AM–6 PM)	39·5 ± 2·2 (86·7)[a]	6·1 ± 0·5 (13·3)[a]	45·6 ± 2·7 (47·4)[b]
12 h night (6 PM–6 AM)	41·8 ± 3·1 (82·5)[a]	8·9 ± 1·3 (17·5)[a]	50·6 ± 4·5 (52·6)[b]
P (day vs night)	NS	< 0·005	< 0·05
24 h	78·7 ± 5·8 (85·0)[a]	13·8 ± 2·0 (15·0)[a]	92·5 ± 7·8 (100)[b]

Each value represents mean ± SD from 6 fishes. NS, Not significant.
[a]Per cent of total ammonia-N and urea-N excreted.
[b]Per cent excretion (total-N) during 12 h day, 12 h night and 24 h.

Table 2. Diurnal variation of urine output (ml/g body wt/12 h) and concentration of ammonia-N and urea-N (μg N/ml) in urine.

		Concentration in urine		
	Urine output	Ammonia-N	Urea-N	Ammonia-N/urea-N
12 h day	0·08 ± 0·01	2·2 ± 0·4	18·5 ± 4·2	0·12
12 h night	0·11 ± 0·01	2·4 ± 0·4 (11%)	20·7 ± 6·4 (12%)	0·12
P (day vs night)	< 0·001	NS	NS	

Each value represents mean ± SD from 6 fishes. NS, Not significant.
Per cent increase of concentration are given in parentheses.

ammoniotelic (purely aquatic freshwater) species than other species mentioned which have ureogenic capacity with functional ornithine-urea cycle.

4. Discussion

Although ammonia-N was found to be the major excretory product like other freshwater teleosts, significant amount of urea-N was also being excreted by *H. fossilis* in its normal aquatic medium (table 1). The ornithine-urea cycle was found to be either incomplete or non-functional in all the freshwater teleosts studied by various workers (Brown and Cohen 1960; Huggins *et al* 1969; Wilson 1973). However, a functional ornithine-urea cycle has been reported in 4 (*H. fossilis, Clarias batrachus, Amphipnous cuchia* and *Anabas testudineus*) out of 5 species of freshwater air-breathing fishes studied (Saha 1986; Saha and Ratha 1987). In all the freshwater and most of the marine fishes excretion of ammonia-N and urea-N ranged between 80–100% and 0–20% respectively (table 4). Gordon *et al* (1969, 1970, 1978) presented a different picture reporting excretion of only about 40% of ammonia-N and 60% urea-N in 3 species of amphibious marine fishes *Periophthalmus sobrinus, Sicyasis sanguineus* and *Periophthalmus cantonensis.* However, Morii *et al* (1978) reported 91% excretion of ammonia-N by one of these species, *P. cantonensis* under identical conditions. Inspite of this controversy in some marine fishes, the freshwater fishes show a definite correlation between their ammonia-N excretion and their ability to convert ammonia to urea (table 4). The excretion of ammonia-N was about 88–100% in those species without having a complete or functional ornithine-urea cycle and below 85% in those species with a functional ornithine-urea cycle. In the latter group including *H. fossilis*, some of the ammonia formed possibly got converted to urea and therefore, the excretion of urea-N was relatively higher. The ratio of ammonia-N : urea-N excretion was about 8–10 in the purely aquatic and 4–6 in the amphibious freshwater fishes. The facultative ureogenic amphibious species such as *H. fossilis* having a functional ornithine-urea cycle did behave like an ammoniotelic purely aquatic species by excreting primarily ammonia-N in its normal freshwater habitat (table 1).

Like other freshwater teleosts almost all the ammonia-N (> 99%) and most of urea-N (~ 75%) were excreted through extra-renal sources in *H. fossilis* (table 3). Excretion of N through extra-renal sources as ammonia was reported to be 90% in carp (Smith 1929; Vellas and Serfaty 1974) and 60–90% in coho salmon (McLean

Table 3. Diurnal pattern of renal and extra-renal excretion of ammonia-N and urea-N (μg N/g fish wt/h) in male *H. fossilis*.

	Renal excretion			Extra-renal excretion			Total excretion		
Duration	Ammonia-N	Urea-N	Ammonia-N: Urea-N	Ammonia-N	Urea-N	Ammonia-N: Urea-N	Ammonia-N	Urea-N	Ammonia-N: Urea-N
12 h day	0·015 ± 0·002 (0·6)	0·12 ± 0·02 (24·6)	0·12	3·3 ± 0·18 (99·4)	0·4 ± 0·06 (75·4)	8·6	3·3 ± 0·2	0·50 ± 0·04	6·6
12 h night	0·022 ± 0·005 (0·6)	0·20 ± 0·07 (26·8)	0·11	3·5 ± 0·3 (99·4)	0·54 ± 0·05 (73·2)	6·4	3·5 ± 0·3	0·74 ± 0·11	4·7
P (day vs night)	< 0·02	< 0·05		NS	< 0·001		NS	< 0·005	
24 h	0·018 ± 0·003 (0·6)	0·15 ± 0·03 (26·5)	0·12	3·3 ± 0·3 (99·4)	0·42 ± 0·06 (73·5)	7·7	3·3 ± 0·3	0·57 ± 0·08	5·7

Each value represents mean ± SD from 6 fishes.
Per cent of nitrogen excretion through renal and extra-renal sources are given in parentheses.
NS, Not significant.

Table 4. Ammonia-N and urea-N excretion by amphibious and fully aquatic fishes immersed in water. Data have been converted into common units (μg N/g body wt/h).

Species	Habitat	Temp (°C)	Ammonia-N	Urea-N	Ammonia-N: Urea-N	References
Amphibious fish						
Periophthalmus sobrinus	Marine	23–30	6·9 (40·6)	10·1 (59·4)	0·68	Gordon *et al* (1969)
Sicyases sanquineus	"	20–23	5·3 (41·4)	7·6 (58·9)	0·70	Gordon *et al* (1970)
Periophthalmus cantonensis	"	20–23	6·3 (46·0)	7·4 (54·0)	0·85	Gordon *et al* (1978)
Periophthalmus cantonensis	"	18–22	4·42 (91·0)	0·44 (9·0)	10·05	Morii *et al* (1978)
Blennius pholis	"	13	1·2 (81·3)	0·3 (18·7)	4·3	Davenport and Sayer (1986)
Channa punctatus	Freshwater	20–22	8·19 (89·1)	1·0 (10·9)	8·19	Roy and Das (1986)
Channa punctatus	"	18–22	4·05 (94·4)	0·24 (5·6)	16·9	Saha (1986)

Anabas testudineus	Freshwater	18–22	5·84 (86·1)	0·94 (13·9)	6·21	Saha (1986)
Clarias batrachus	"	"	5·92 (85·0)	1·04 (15·0)	5·69	"
Amphipnous cuchia	"	"	2·37 (83·7)	0·46 (16·3)	5·15	"
Heteropneustes fossilis	"	"	3·28 (85·0)	0·58 (15·0)	5·75	Present study
Purely aquatic						
Platichthys stellatus	Marine	12	2·40 (88·2)	0·32 (11·8)	7·5	Wood (1958)
Opsanus tau	"	22	3·77 (100)	trace		Read (1971)
Potamotrygon spp.	Freshwater	26–28	4·54 (88·3)	0·62 (11·7)	7·32	Gerst and Thorson (1977)
Cyprinus carpio	"	18·5	4·51 (90·7)	0·46 (9·3)	9·80	Smith (1929)
Cyprinus carpio	"	20	10·73 (88·2)	1·43 (11·8)	7·50	Pequin and Serfaty (1966)

Per cent of excretion are given in parentheses.

and Fraser 1974). In rainbow trout, *Salmo gairdneri* only about 3% of the total N was excreted in the urine and over 60% of the total N excreted was in the form of ammonia (Fromm 1963). Besides the gills, extra-renal excretion of N by the skin has been reported in two mudskipper fishes, *P. cantonensis* and *B. pectinirostris* (Morii *et al* 1978) and an air-breathing marine fish *Blennius pholis* (Davenport and Sayer 1986). The smooth scaleless mucoid skin of *H. fossilis* might have also served as an efficient excretory surface for extra-renal excretion of N along with the gills.

More urine output and excess N excretion in the night by *H. fossilis* (table 2) give an indication of the occurrence of diurnal variability in N excretion in this fish. Circadian variability in N excretion could not be observed in a freshwater sockeye salmon (*Onchorhynchus nerka*) under starved condition (Brett and Zala 1975). We have not come across any report on the circadian pattern of N metabolism or excretion in freshwater air-breathing fishes. King and Goldstein (1983) reported increase in urine flow due to an increased glomerular filtration rate and thus increase in renal ammonia excretion under acidotic condition in gold fish, *Carassius auratus*. Increased metabolism at night might have caused over production of some acidic metabolites resulting in metabolic acidosis in *H. fossilis*. This might have induced significant increase in urine flow and renal excretion of both ammonia-N and urea-N during night than day (table 1). Garg and Sundararaj (1986) suggested that *H. fossilis* was nocturnally active. Enhanced activities of acetylcholinesterase and tyrosine transaminase suggesting higher metabolism during night than day was also reported in *H. fossilis* (Ramanujam *et al* 1981). Renal ammonia-N excretion was very low (< 1% of total ammonia-N excretion) (table 3) to be considered for any greater physiological significance. However, both renal and extra-renal urea-N excretion were significantly higher during the night than day in *H. fossilis* (table 3). Increased amino acid metabolism might have produced ammonia in excess besides the keto acids resulting into increased urea synthesis by the fish at night.

The results indicate that the freshwater air-breathing teleost, *H. fossilis* does behave like purely aquatic species in excreting primarily ammonia-N as nitrogenous waste in aquatic environment. However, higher quantities of urea-N excretion makes it unique perhaps due to the presence of a functional ornithine-urea cycle, converting some amount of ammonia to urea.

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