Sudden transfer of freshwater catfish, *Clarias batrachus* to media of various salinities ranging from 10-100% sea water (SW) establishes that it is stenohaline fish with upper limit of tolerance up to 30% SW (298 mmol/kg) which gets marginally elevated upon its acclimation in sub-lethal salinities. No appreciable change in plasma osmolarity following transfer of the catfish to 15% SW (132 mmol/kg) even after 14 days reveals that it can actively osmoregulate up to 15% SW. Unlike other teleost, this fish lacks the bimodal adaptive and regulatory phases as evident from the increase in the plasma osmolarity within 0.5 h of transfer to higher salinities which remained elevated throughout the experiment. The changing profile of plasma cortisol following transfer to higher salinities suggests the involvement of this hormone in SW adaptation.

INTRODUCTION

The aquatic animals particularly the fishes living in a highly divergent environment ranging from salt-rich sea water (SW) to salt-deficient freshwater (FW) have to continuously cope up with the osmotic variability of the ambient environment. Studies on the physiology of osmoregulation of aquatic animals particularly those of fishes are vitally important since they provide high nutritive and easily digestible source of protein.

Of the large number of fish species inhabiting Indian subcontinent, there is virtually no information on the physiology of osmoregulation of any teleost fish with the exception of Asian catfish, *Heteropneustes fossilis* (Goswami et al., 1983; Parwez and Goswami, 1985; Parwez et al., 1979, 1984, 1994, Sherwani, 1998; Sherwani and Parwez, 2000). However, results of the study on *H. fossilis* have clearly revealed species-specificity in the osmoregulatory mechanism of the teleost fishes in hetero-osmotic environment. Thus, detailed studies on large number of fishes are clearly warranted to gain an insight of such diversities. With this objective in view, in the present study, an attempt has been made to investigate the osmoregulatory physiology of an economically important catfish, *C. batrachus* which is found in native FW of Indian subcontinent.
Hence, the present study is designed to (i) establish whether this fish is stenohaline or euryhaline by determining its upper salinity tolerance limit, (ii) determine whether survival of this fish up to its upper salinity tolerance limit is due to active osmoregulation or by passive tissue tolerance, (iii) find out whether this fish has bimodal adaptive and regulative phases and (iv) investigate the role of cortisol (F), the principal corticosteroid, during the adaptation of the fish in different salinities.

**MATERIAL AND METHODS**

**Collection and care of fish**

Adult specimen of catfish, *C. batrachus* weighing 40-50 g were obtained from local fish market of Aligarh and acclimated to laboratory conditions (temperature 25 ± 2°C, photoperiod 12 L : 12 D) for 15 days in glass aquaria (60 x 25 x 30 cm) containing stored dechlorinated tap water (TW). During this period they were fed daily *ad libitum* with Hindleever laboratory animal feed (Hindustan Lever Limited, Bombay, India) and the water in the aquaria was renewed daily by siphoning off and replenishing simultaneously with FW adjusted to laboratory temperature.

**Artificial SW**

Artificial SW was prepared in dechlorinated TW according to Goswami *et al.* (1983) and various concentrations of SW were prepared by diluting full strength artificial SW with dechlorinated TW.

**Plasma samples**

Blood was drawn from caudal artery into heparinized glass syringes fitted with 24-gauge disposable needles. Immediately after collection, the blood was centrifuged for 10 min at 3000 rpm (Remi Ltd., India, model no. R8C) and plasma separated and stored at -20°C until analyzed.

**Estimations**

**Plasma osmolarity**

Plasma osmolarity was measured in 10 μl sample with Vapour Pressure Osmometer (Wescor 5500, Utah, USA) and expressed as mmol/kg.

**Plasma F**

Plasma F levels were estimated by radioimmunoassay (RIA) employing Biodata F Bridge Kit (Code 14394 manufactured by Biodata S.P.A. Montecelio) which measures F over the range from 10 to 1000 ng/ml.
Statistical analysis

Data for all parameters were expressed as mean ± standard error. Statistical comparisons between experimental and control groups were made by students’ $t$-test (Snedecor and Cochran, 1971).

**Experiment 1**
Groups of fish were transferred from TW to glass aquaria containing various dilutions of SW ranging from 10 to 100%. The fish were fed on alternate days and water was replenished daily with the same dilutions of SW. Fish were observed daily and the mortality, if any, was recorded.

**Experiment 2**
The upper salinity tolerance was determined after prior acclimation of the fish in sub-lethal salinities. In this experiment fish maintained in 10, 15, 25 and 30% SW were transferred to their next higher salinities i.e. 15, 25, 30, and 35% SW, respectively. The fish were fed on alternate days and the media replaced daily. Fish were observed daily and mortality was recorded. After 14 days, all the fish kept in different salinities were bled for the estimation of plasma osmolarity and F.

**Experiment 3**
In this experiment, fish were maintained in 30 and 35% SW and TW maintained group served as control. The fish in all the three groups were subjected periodically to handling to eliminate stress factor during sampling. Five fish from each group were sampled at ½, 3, 8 and 24 h and 4, 8 and 14 days post transfer. The blood was collected from the caudal artery as described earlier and the plasma thus collected was utilized for the estimation of plasma osmolarity.

**RESULTS**

**Experiment 1**
No mortality was observed in fishes transferred directly to 10, 20 and 30% SW up to 7 days when the experiment was terminated (Table 1). However, in 35% SW, more than half of fishes died within 72 h, mortality being heaviest during the last 24 h and the remaining fish died by 7 days. Mortality at higher concentrations (40, 50, 65 and 100% SW) was so swift and complete that no fish survived beyond the first 24 h (Table 1).

Upon transfer to lethal salinities, the fish became sluggish and settled at the bottom of the aquarium. After a few spasmodic excursions to the surface of water, the fish lost balance and became unresponsive to tactile stimuli. Death, indicated by cessation of opercular movements and heart beat, followed soon. The dead fish as well as survivors at the end of the experiment were autopsied and the gut examined. There
was no evidence of the fish having swallowed significant quantity of water in any of the groups.

Table 1. Mortality rates following abrupt transfer of freshwater teleost, *Clarias batrachus* to various concentrations of artificial sea water (SW) (No. of fish in each salinity = 7)

<table>
<thead>
<tr>
<th>Medium (%SW)</th>
<th>Osmolarity (mmol/kg)</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>997</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>65</td>
<td>667</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>493</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>394</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td>320</td>
<td>0</td>
<td>14</td>
<td>42</td>
<td>44</td>
</tr>
<tr>
<td>30</td>
<td>298</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>265</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>132</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Experiment 2

Salinity tolerance

In this experiment, the fish preadapted in 10, 15 and 25% SW for 7 days in each salinity and transferred to 15, 25 and 30% SW respectively, no mortality was observed even after 14 days (Table 2). However, in the 35% SW, that received the fish from 30% SW, after acclimation for 7 days, 14% mortality each was observed on 3rd, 9th and 12th day following transfer and all the fish died in this salinity within next two days, the mortality being heaviest on 13th day.

Table 2. Mortality rates following gradual transfer of freshwater teleost, *Clarias batrachus* to various concentrations of seawater (SW) (No. of fish in each salinity = 7)

<table>
<thead>
<tr>
<th>Medium (SW)</th>
<th>Osmolarity (mmol/kg)</th>
<th>Per cent mortality at (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35% (Transferred from 30% after 7 days)</td>
<td>366</td>
<td>14</td>
</tr>
<tr>
<td>30% (Transferred from 25% after 7 days)</td>
<td>320</td>
<td>0</td>
</tr>
<tr>
<td>25% (Transferred from 5% after 7 days)</td>
<td>291</td>
<td>0</td>
</tr>
<tr>
<td>15% (Transferred from 0% after 7 days)</td>
<td>136</td>
<td>0</td>
</tr>
</tbody>
</table>
Plasma osmolarity

In 15% SW, there was no appreciable change in plasma osmolarity even after 14 days (Fig. 1). However, in the group of fish transferred to 25% SW, plasma osmolarity increased significantly ($p < 0.01$) over the TW control which got further accentuated in the fish transferred to 30% SW ($p < 0.001$).

Plasma F

A perusal of Fig. 2 reveals that the transfer of the fish to 15% SW resulted in a marked elevation (31% over the control) in the titers of plasma F. Interestingly, in still higher salinities, plasma F levels registered a gradual decline so much so that in 30% SW it became markedly lower than TW control (Fig. 2).

**Experiment 3**

Plasma osmolarity

When the fish were transferred from TW to 30 and 35% SW, the plasma osmolarity increased markedly within $\frac{1}{2}$ h of transfer by 12 and 7%, respectively over their respective TW controls (Fig. 3). In 30% SW, plasma osmolarity exhibited a steep rise up to 8 h, stabilizing thereafter at 24 h, becoming more or less constant from 8 days to 14 days at the level of 333 mmol/kg. These levels are, however, still significantly higher compared to their TW control (Fig. 3).
DISCUSSION

Literature abounds with large number of studies to establish that osmotically more conservative teleost fish, rightly termed as stenohaline, can withstand upper salinity tolerance almost close to their body fluid osmolarity. This is amply demonstrated in channel catfish, *Ictalurus punctatus* having upper salinity tolerance limit up to 12 g/l (34% SW) (Allen and Avault, 1969; Stickney and Simco, 1971; Perry, 1973), *Clarias lazera* up to approximately 26% SW (Clay, 1977; Chervinski, 1984) and the Indian catfish, *H. fossilis* up to 35% SW (Parwez et al., 1979). However, the common carp, *Cyprinus carpio* has the highest tolerance up to 1.5% salt water (about 45% SW) (Hegab and Hanke, 1982). Hence, the present observation on the air-breathing teleost, *C. batrachus* clearly establishes that it is a stenohaline fish whose upper limit of tolerance up to 30% SW is in good agreement with what is mostly documented in the literature for other stenohaline teleost.

It has been seen in certain cases that preadaptation in sublethal salinities often enhances the upper tolerance limit of the fish. However, only a marginal change has been observed in the present study inasmuch as following abrupt transfer of *C. batrachus*, its tolerance up to 35% SW gets enhanced from 7 days to 14 days. Similar absence of the effects of prior acclimation on survival in higher salinities has also been reported for the channel catfish (Allen and Avault, 1969; Stickney and Simco, 1971; Perry, 1973; Norton and Davis, 1977) and young catfish, *Clarias lazera* (Chervinski, 1984) and the Indian catfish, *H. fossilis* (Parwez et al., 1979). In contrast, the mature catfish, *C. lazera*, with pre-acclimatization, show a dramatic increase in upper salinity limit from a mere 26% SW following direct transfer to 20% (about 51.3% SW) following preadaptation in sub-lethal salinities (Clay, 1977).

A perusal of results reveals that *C. batrachus* actively osmoregulates in the salinities up to 15% SW. The studies of Hegab and Hanke (1986) also demonstrate that there is approximately 10% increase in plasma osmolarity in carp, *C. carpio* following its transfer to 1.5% salt water (about 45% SW). However, in *I. punctatus* active osmoregulation is evident until the ion concentration of the medium exceeds that of the plasma osmolarity of normal FW fish (Norton and Davis, 1977). The increase in plasma osmolarity in 30 and 35% SW in *C. batrachus* showed that the survival of this fish in salinities higher than 15% SW is due to passive tissue tolerance. This is in contrast to the euryhaline fishes such as flounder, salmon and eel, which upon transfer even up to 100% SW remain hypoosmotic to the medium (Bentley, 1971). Transfer of the most euryhaline fishes to SW is accompanied by a series of physiological events demarcated into an adaptive (Houston, 1959) and a regulative phase. During the adaptive phase, there is an abrupt increase in plasma osmolarity, electrolyte concentration, water content, sodium and chloride space and muscle electrolyte concentration. This is followed by a chronic
 regulative phase during which various parameters return to normal levels and are established at a steady state (Holmes and Donaldson, 1969; Holmes and Lockwood, 1970). In the present investigation, the significant increase in plasma osmolarity within 8 h following transfer to 30 and 35% SW suggests that this fish does enter into initial adaptive or adjustive phase. Similar type of adjustive phase was also evident in S. gairdneri (Houston, 1959), H. fossilis (Goswami et al., 1983) and I. punctatus (Eckert et al., 2001). In C. batrachus, the adjustive phase leads to the enhancement of plasma osmolarity to the tune of 11% in 30% SW which is close to what has been observed in carp (Hegab and Hanke, 1982). However, the persistently high plasma osmolarity values in 30 and 35% SW suggest the lack of a regulative phase. A similar lack of regulative phase, though in reverse direction, has been reported in the barred surfperch, Amphistichus argenteus, a marine stenohaline teleost, which shows a continuous decline in plasma osmolarity and plasma sodium and chloride concentrations when transferred from 100% SW to dilute SW (Holmes and Lockwood, 1970).

There are overwhelming number of studies confirming hormonal control of osmoregulation in teleost fishes. Of the vast array of hormones, F is considered to be the principal hormone concerned with hydromineral balance in higher salinities (Hegab and Hanke, 1984; Madsen et al., 1994; Eckert et al., 2001; McCormick, 2001). There is increasing evidence to show that plasma F levels in teleost exhibit a significant increase following transfer to higher salinities (Hegab and Hanke, 1984; Madsen et al, 1994; Eckert et al., 2001). Also in the present study, plasma F levels were increased when the catfish were transferred to 15 and 25% SW. However, there was a marked decline in plasma F levels when the catfish were transferred to 30% SW which may probably not be due to the reduced production but rather to enhanced utilization and clearance (Hirano et al., 1976; Goswami et al., 1983; Sherwani, 1998). Plasma F levels were also decreased when the catfish, H. fossilis were transferred to 30% SW (Goswami et al., 1983).

In conclusion, the present study on air-breathing teleost, C. batrachus has unequivocally established that it is a stenohaline fish with the upper salinity tolerance limit up to 30% SW (298 mmol/kg) which gets only marginally elevated upon its acclimation in sublethal salinities. The study has also shown that this fish actively osmoregulates up to 15% SW (132 mmol/kg) beyond which its survival up to its upper salinity limit is merely by passive tissue tolerance. This fish, unlike other teleost, lacks bimodal regulatory and adaptive phases. However, like other teleosts, in C. batrachus F influence the osmotic adjustment after transfer of the catfish to higher salinities.

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