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# Osmoregulation in Mudskippers : Effect of Salinity Stress on Blood Ionic Concentrations in the Mudskipper, *Periophthalmus dipus* (Cuv & Val)

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The natural habitat of the amphieuryhaline fish, *Periophthalmus dipus* (Cuv & Val), lies usually in a dynamic state of fluctuating salinities. The mudskipper, *P. dipus* when subjected to lower salinities, showed notable response by showing significantly lower concentrations of $Na^+$ and $Cl^-$ ions in blood. $Ca^{2+}$ ion level was found to be lower in the animals acclimated to 60% SW, but the animals were found to be efficient in $Ca^{2+}$ regulation. The stress induced changes in plasma electrolytes suggest that the mudskippers, when subjected to low salinities, undergo blood ionic changes, initially by an outflux of $Na^+$ and $Cl^-$, showing that *P. dipus* is a poor regulator of $Na^+$ and $Cl^-$ ions in the blood. Mudskippers show an 'evassive adaptation' to their environment during abrupt changes in temperature and salinity. Poor regulation of $Na^+$ and $Cl^-$ ions in the blood may be one of the causating factors for such adaptation.

**Key Words: Osmoregulation, Mudskippers, Ion fluctuations, Evasive adaptation**

## Introduction

Mudskippers are amphieuryhaline fishes, build burrows on the mudflats of estuaries. The microenvironment within these burrows is usually different from that of the estuary and their terrestrial environments have wide diurnal and seasonal changes in temperature (Sivaram Prasad 1989). Their habitat and territorial behaviour were studied on the mudflats of Kuwait (Tytler & Vaughan 1983, Clayton & Vaughan 1986, Clayton 1987).

There are evidences to indicate regulation in the individual ionic concentrations of the body fluids of fishes against the imposed osmotic stress and in most of these studies, ions, like $Cl^-$, $Na^+$, $K^+$, $Ca^{2+}$ and to some extent $Mg^{2+}$, were found to be significant. It has been shown that $Na^+$, $Cl^-$ and $K^+$ are independently regulated (Gilles 1979). Very few physiological studies have been carried out on different species of Mudskippers (Lee et al. 1987, 1991, Ip et al. 1990, 1991a,b, Low et al. 1988, 1990, Sivaram Prasad 1989, Ip & Low 1990).

In this paper we present our results on physiological changes in *P. dipus* to salinity stress, by studying the effect of different sea water(SW) concentrations on blood ionic levels ($Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$) maintained under laboratory conditions, with an attempt to find their adaptations to their natural environment.

## Materials and Methods

### *Collection of Animals*

Mudskippers, *P. dipus* were caught by hand, from the mudflats of Jodia situated on the Saurashtra coast, India. The animals were brought to the laboratory in plastic troughs containing only sea water to 3 cm. level.

### *Acclimation to the Laboratory*

Healthy animals, weighing 25 ± 4 g were

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transferred to cement troughs containing mud and natural sea water from sea coast. The animals fed on the algae grown on the mud and the walls of the troughs, alongwith the organic debris and arthropods like *Artemia* etc., available in the medium. They were allowed to have the same light and dark periods as in their natural habitat. Laboratory temperature was observed to be in the range of 25-30 °C within their micro environment. The animals were kept for acclimation to the above mentioned semi natural conditions for a week.

*Experiment*

Different concentrations of natural sea water were made and used as external stress media. Natural sea water brought from Jodia coast(Arabian Sea) has been diluted with deionized water to make 20% (8.22 ‰ ± 0.23), 40% (14.25 ‰ ± 0.12), 50% (18.20 ‰ ± 0.2), 60% (23.85 ‰ ± 0.3), 80% (30.27 ‰ ± 0.14), 100% (36.01 ‰ ± 0.18) and 0% (0.16 ‰ ± 0.11) SW (i.e. deionized water). The medium was replaced every 24 hr. Salinity of each stress medium was measured as per Tait(1972).

Among the various size groups of *P. dipus* available, the adults, measuring 12 ± 2 cm, weighing 25 ± 5 g were selected, without differentiating the sex. Atleast 6 animals were exposed to each stress medium for 3 days.

The fish were starved during the experiment to nullify the effect of differential feeding. The experiments were repeated atleast thrice. Control animals were kept in 50% SW with a liberty to be in or out of water by keeping the trough at a slanted position. They were stunned by mechanical shock for sample preparation and analysis. Blood was pooled from all animals exposed to the same treatment by directly puncturing the heart and drawn with a tuberculin syringe fitted with 22 No. needle. It was transferred into graduated heparinized centrifuge tube and was centrifuged for 5 min. at 300 rpm in a clinical centrifuge. Analysis of $Na^+$, $K^+$, $Ca^{2+}$ in blood plasma was carried out by Flame photometry. Chloride estimation was carried out according to Schales and Schales (1941), by titrating the sample against $HgNo_3$ after adding 0.06 ml of Diphenyl carbazone indicator.

Temperature and salinity changes were monitered throughout the year, by taking monthly readings at the site of collection of animals.

**Results**

The laboratory acclimated animals when transferred to different test salinities, immediately showed hyper activity by erratic jumping followed by restless swimming. Within an hour or two, the fish ceased to show such unusual behaviour. After 48 hr, sluggishness set in their behaviour. After 6-12 hr of their sluggish behaviour, they almost stopped moving around. The buccal movement was observed to be faster than in the control fish, showing excessive buccal respiratory activity, indicating an excessive oxygen demand.

At Jodia coast, temperature and salinity monitered throughout the year showed considerable variation. Air temperature ranged from 18 to 32°C in riverine area and 17 to 34°C in estuarine area. Water temperature ranged from 19 to 30°C in riverine area and 19 to 32°C in estuarine area. Salinity ranged from 1 to 18 ‰ in riverine area and 2 to 23 ‰ in estuarine area. It was observed that during high tide everyday and during run off water in monsoon season, the salinity and temperature changes abruptly, and suddenly the mudskippers disappear from the surface and take shelter in their burrows.

Highest concentration of $Na^+$ was found in fish maintained in 100% SW and the lowest concentration was in fish maintained in 0% SW. An increasing trend in $Na^+$ concentration was shown in the range of 0% to 100% SW, with an exception of 80% SW(table 1). The regression analysis showed a direct positive relationship between $Na^+$ concentration in

**Table 1** ***Periophthalmus dipus*** *: showing ionic changes after 3 days of exposure to different seawater concentrations. Values expressed as m.eq $l^{-1}$ are mean of 6 individual observations*

| | % Sea water concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 20 | 40 | 50 | 60 | 80 | 100 | F |
| $Na^+$ | 61.62 ±23.7 | 113.6 ±48.63 | 155.06 ±49.99 | 180.88 ±13.22 | 185.04 ±63.03 | 121.3 ±67.03 | 220.99 ±121.18 | 2.489* |
| $K^+$ | 11.75 ±3.78 | 9.2 ±2.6 | 10.13 ±2.04 | 10.52 ±2.44 | 10.78 ±2.38 | 8.87 ±2.03 | 7.396 ±1.07 | 0.195 |
| $Ca^{2+}$ | 9.43 ±1.05 | 9.57 ±2.35 | 10.03 ±2.1 | 9.26 ±1.81 | 8.07 ±1.69 | 9.74 ±0.69 | 10.11 ±2.52 | 0.194 |
| $Cl^-$ | 56.59 ±2.15 | 129.16 ±3.37 | 133.10 ±10.21 | 146.51 ±34.08 | 156.24 ±52.16 | 160.21 ±54.26 | 173.41 ±57.34 | 3.314* |

*$P < 0.05$

blood and salinity of external medium of the order Y = 92.06 + 1.32X. The value of correlation coefficient (r = 0.46) was significant at P < 5%.

Concentration of potassium was the highest in animals under 0% SW(table 1). The amount of blood potassium showed a negative trend with external SW. The relationship was of the order Y = 10.27-0.002X and the value of correlation coefficient(r) was nonsignificant.

The concentration of $Ca^{2+}$ was highest in the fish from 100% SW. and the lowest in those from 60% SW(table 1). Calcium ion concentration in the blood of the animals was found to be increasing with an increase in external salinity and the relationship was of the order Y = 9.26 + 0.003X. and the value of correlation coefficient (r) was nonsignificant.

Blood chloride in *P. dipus* was highest in the fish from 100% SW, and the lowest in the fish maintained in 0% SW(table 2). Chloride concentration increased in the blood with increasing salinity. The relationship was of the order Y = 93.39 + 0.9X. The value of correlation coefficient (r = 0.59) was highly significant at P < 1%.

## Discussion

After acclimation for 3 days in 0% SW ) i.e. deionized water) *P. dipus* showed lower concentration of $Na^+$ which was significant at P < 5%. Loss of sodium, probably, into the external medium could be due to passive diffusion along the gradient. Such loss of sodium to the external medium was also found in the fish acclimated to other salinities.

Earlier studies on other euryhaline fish, *A. anguilla, Fundulus kansae, Platichthys flessus* and *Salmo salar* showed sodium concentration in blood plasma was found to be low, when in fresh water, or lower salinities and these studies led to the conclusion that sodium fluxes in marine fishes can be related to "exchange diffusion" which may play an important role during their transfer to diluted media (Gilles 1979). However, branchial exchange diffusion is not confined to, or present in, all the euryhaline fishes. On the contrary, the secondary "delayed regulation" mechanism, whereby the gill permeability is gradually restricted, after the transfer of fish to fresh water, has only been described for euryhaline species(Gilles 1979). Similarly, when the mudskipper, *P. dipus* was exposed to lower salinity showed lower concentration of blood sodium, which

is in agreement with the studies of Lee et al (1987) on *P. chrysopilos*.

Low $K^+$ concentrations were reported in the blood plasma of *A. anguilla, Platichthys flesus, S. salar*, when the fish was in fresh water and on the contrary, high $K^+$ concentrations were reported in blood plasma of *Fundulus kansae* when the fish was in fresh water (Gilles 1979). However, from the present results, the correlation coefficient (r) was nonsignificant and hence, the variation in the absolute values of $K^+$ concentration were considered to be of insignificant. Thus, it is inferred that *P. dipus* can regulate blood $K^+$ concentrations even after 3 days of exposure to low saline media.

$Ca^{2+}$ concentrations in the blood plasma of *P. dipus* decreased when they were maintained in low salinities for 3 days (table 1), and this decrease was nonsignificant. When transferred to fresh water, low calcium concentrations were reported in blood plasma of *A. anguilla* and *Platichthys flesus*, whereas, the $Ca^+$ concentrations were higher in blood plasma of *Fundulus kansae* and of *Salmo salar* (Gilles 1979). However, the loss of $Ca^{2+}$ was insignificant as the correlation coefficient(r) obtained was nonsignificant and hence it can be interpreted as due to an efficient $Ca^{2+}$ regulation of the species.

$Cl^-$ concentration in the blood plasma of mudskippers, showed an increasing trend with increasing salinity of the external medium (table 1). Fresh water fish are unable to produce a salt free urine, even when starvation prevents them from acquiring salts from their food, but they are thought to be able to acquire chlorides from the surrounding medium by means of a mechanism in the gills, even if the ambient concentration of chloride is low (Krogh 1937). Motais et al. (1965) observed that when *P. flesus* was transferred from sea water to fresh water, there is an immediate reduction (about 85%) in the elimination of sodium and chloride by a mechanism located in the gills. However, *Serranus scriba*, which dies if the salinity is changed, does not possess this mechanism. It is clear through the present study, that there will be loss of blood plasma $Cl^-$ when *P. dipus* are maintained in lower salinities for 3 days. The strong positive correlation ($P < 1\%$) found between the blood plasma chloride and external sea water concentrations supports this point. The loss of blood plasma ions of mudskippers in lower salinities is an indication of poor salt regulation.

As the natural habitat of the animals, experimented upon in the present study, lies usually in a dynamic state with fluctuating salinities, there should be some regulatory mechanism operating in the animals to suit to the altered environment. It can be either through a passive process like diffusion or through an active process involving an active uptake of salts, and other morphological adaptations like an increase in the number of glomerulii which makes them cope up with the altered environment (Parvatheswera Rao 1970, Ip et al. 1991b). Four compensatory devices, viz., escape, reduction, regulation and adaptation are available to counteract the detrimental effects of such stress (Kinne 1966). The erratic movement exhibited by the mudskippers on their transfer to different SW concentrations is probably an indication of their 'evassive adaptation' which they successfully accomplish in their natural habitat (i.e. by taking shelter in their burrows during high tide every day and during run off water in monsoon season). From the present results, it is inferred that the mudskippers, when subjected to low saline media, undergo blood ionic changes, initially by an outflux of $Na^+$ and $Cl^-$. Hence, it is inferred that *P. dipus* is poor in $Na^+$ and $Cl^-$ regulation. Thus it is shown that mudskippers being poor in ionic regulation, has to cope up with their environment by evassive adaptation i.e. by taking shelter in their burrows wherein there won't be any abrupt changes in temperature as well as salinity.

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