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Osmoregulation of some fishes from the Bolgoda estuary

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Abstract

The three species of fish studied here, *Etroplus maculatus, Etroplus suratensis* and *Puntius filamentosus* are teleosts indigenous to Sri Lanka. The three species are found in abundance in the Bolgoda estuary and specimens for the present study were obtained from this estuary. The salinity of the lagoon water varies from fresh water to 100% sea water in the different parts of the estuary and fluctuates periodically. Although these fishes are commonly found in both fresh and brackish waters, no work has been reported in respect of their osmoregulatory abilities and salinity tolerances.

This paper reports on some aspects of osmoregulation in *E. maculatus, E. suratensis* and *P. filamentosus.* The two *Etroplus* species were able to tolerate salinities ranging from 0-60% sea water, while *P. filamentosus* was able to tolerate only the narrow range from 0-20%sea water. In all species, sodium salts accounted for most of the osmotic concentration, which is indicative of an evolution in fresh water. Metabolic rates were measured for each group of fish in various salinities to determine the relationship between the medium osmotic concentration and the osmotic work done. *E.maculatus* showed a significant reduction in energy expenditure at 30% sea water, the concentration isosmotic to its plasma osmotic concentration . *E.suratensis* and *P. filamentosus* showed no significant changes in metabolic rates at the different salinities tolerated by them. Salinity preferances were determined for the three species using a horizontal salinity gradient chamber. The *Etroplus* species were found to prefer salinities ranging from 10-20% sea water, while *P. filamentosus* was found to prefer fresh water.

Key words: Salinity tolerance, salinity preference, osmotic regulation

1. Introduction

The three species of fish studied here, *Etroplus maculatus, Etroplus suratensis*, and *Puntius filamentosus* are teleosts indigenous to Sri Lanka. Of them *E.maculatus* and *E. suratensis* bleong to the family Cichlidae and are commonly found in estuarine systems, lagoons and reservoirs. They are popular and commonly available food fishes. Today they are heavily utilized by aquarium fish exporters.*P. filamentosus* belong to the family Cyprinidae and occurs in a wide variety of still and flowing waters in most parts of the country. Small specimens of this species are exported in the aquarium trade. Larger specimens are used for food or as bait (Pethiyagoda, 1991).

The three species of fish are found in abundance in the Bolgoda estuary, situated in the western coastal area of Sri Lanka. Salinity of the estuary water varies from fresh water to 100% sea water in the different parts of the lagoon and fluctuates periodically due to rains and tidal flow from the sea. Although these fishes are commonly found in both fresh and brackish waters, no work has been reported in respect of their osmoregulatory abilities and salinity tolerances.

An understanding of the biological mechanisms and contributing factors leading to adaptation of fish groups to various salinity conditions is of interest for both fundamental and applied reasons. For instance, animals prefer salinities where their physiological mechanisms are properly maintained.In practi cal aquaculture, providing such conditions can promote the growth of the fish and even reduce the incidence of diseases. At the same time growing these fish artificially may help prevent the threat of overexploitation of the naturally occurring fish stocks. This paper reports on some aspects of osmoregulation in *E.maculatus, E.suratensis* and *P.filamentosus*.

2. Materials and Methods

Fishes for the study were obtained from fishermen operating in the Bolgoda estuary. The size classes of fishes taken for the experiments were as follows: *E. maculatus*, 5-6 cm ; *E. suratensis*, 5-8cm: *P. filamentosus*, 6-8cm. Fishes were kept in dechlorinated, aerated fresh water in the laboratory and were fed with commercially available pelleted fish food.

For the determination of salinity tolerance, a range of sea water concentrations from 0-100% sea water with gradual increments of 10% sea water were made up with dechlorinated tap water. These concentrations corresponded to salinities of 0, 3.4 ppt, 6.9ppt, 13.8ppt, 17.25ppt,20.7ppt,24.15ppt,27.6ppt, 31.05ppt and 34ppt respectively. The salinities were checked using a hand refractometer. Batches of fish of each species were gradually acclimated to higher salinity levels by keeping them for 2 weeks in each salinity level before transferring them to the adjoining higher salinity level.Mortality of fishes in each salinity level was recorded with time and mortality percentages of fishes in each salinity level was calculated with time.

Blood samples were collected from each species (n=5) at the end of the acclimation period in each salinity level. At the lethal salinities the blood samples were taken soon after their deaths. A fish was anaesthetized (Ms-222) and blood from the heart was obtained into heparinized capillary tubes.Blood collected was centrifuged at 11000 rpm for 5 minutes (Hawksley haematocrit centrifuge) to separate the plasma. Osmotic concentration and Na^+ concentration of plasma and that of the external medium were measured using respectively an osmometer (Wescor 5100 c) and a Flame photometer (Corning 400).

Energy expenditure during osmoregulation was determined by measuring the oxygen consumption rates of each species at the various salinity levels, using a flow through respirometer (Hoar and Hickman,1975). Metabolic rates were expressed as oxygen consumption per unit weight of fish per hour(n=5).

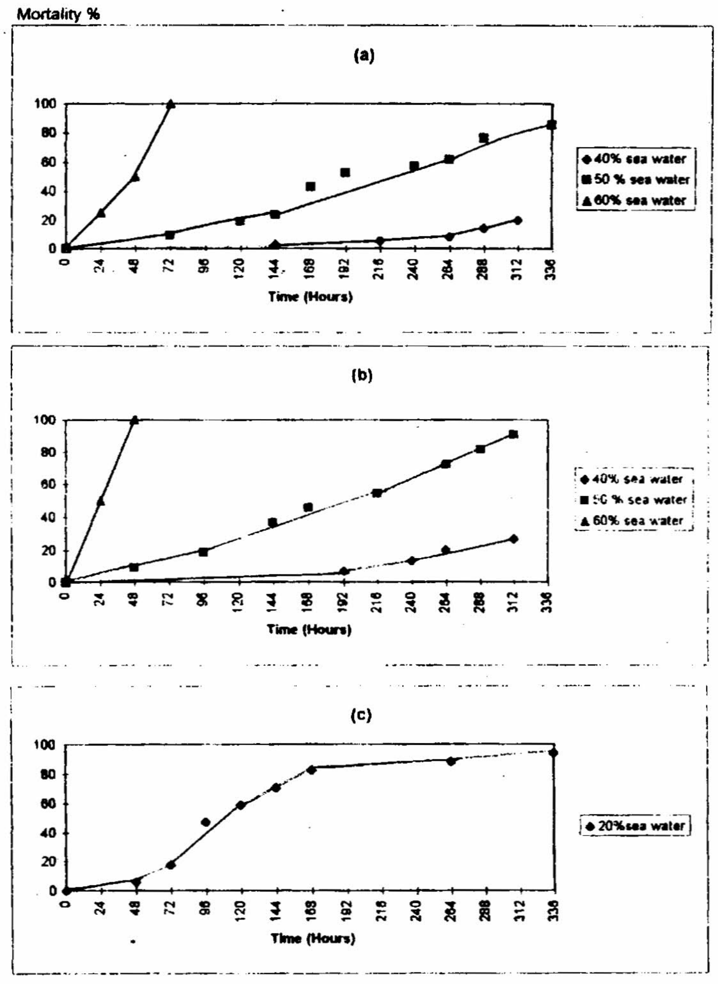
Statistical comparisons were made using the student' s t test at the 5% level of significance. values obtained in fresh water were considered as the control values.

Salinity preferences of each species were determined using a horizontal salinity gradient chamber (Audet et at, 1986; Miller et al, 1983 Fivizzani and Meier 1978). The location of a fish in the chamber was recorded every 5 minutes for a the period of 4 hours. Eight replicates were carried out for each fish species. Control experiments were conducted in the same way but without a salinity gradient. The Chi Square test was applied to determine if the position of the fish species in the test chamber was influenced by salinity. When the overall test was significant, preference or avoidance reactions of a species at each salinity level was determined using the student's t test.

Ressults

salinity tolerance

The incipient lethal salinities after gradual acclimatization were 40% sea water (13.6ppt) for the two *Etroplus* species (Fig. 1*a*b) and 20% sea water (6.9ppt) for *P. filamentosus* (Fig.1c). The percentage mortalities at these salinity levels were 20% for *E. maculatus,* 27%for *E.suratensis* and 94% for *P. filamentosus.* 14.3% of *E.maculatus* and 9.1% of *E. suratnsis* were able to survive in 50% sea water level for the period of two weeks. In 60% sea water *E. maculatus* and *E.suratensis* were unable to last for more than 72 hours and 48 hours respectively.



Figre 1 Mortality Percentages of (a) *Etroplus maculatus* (b) *Etroplus Suratensis* and (c) *Puntius filamentosus* in different salinty media.

salinity effects on osmoregulatory parameters

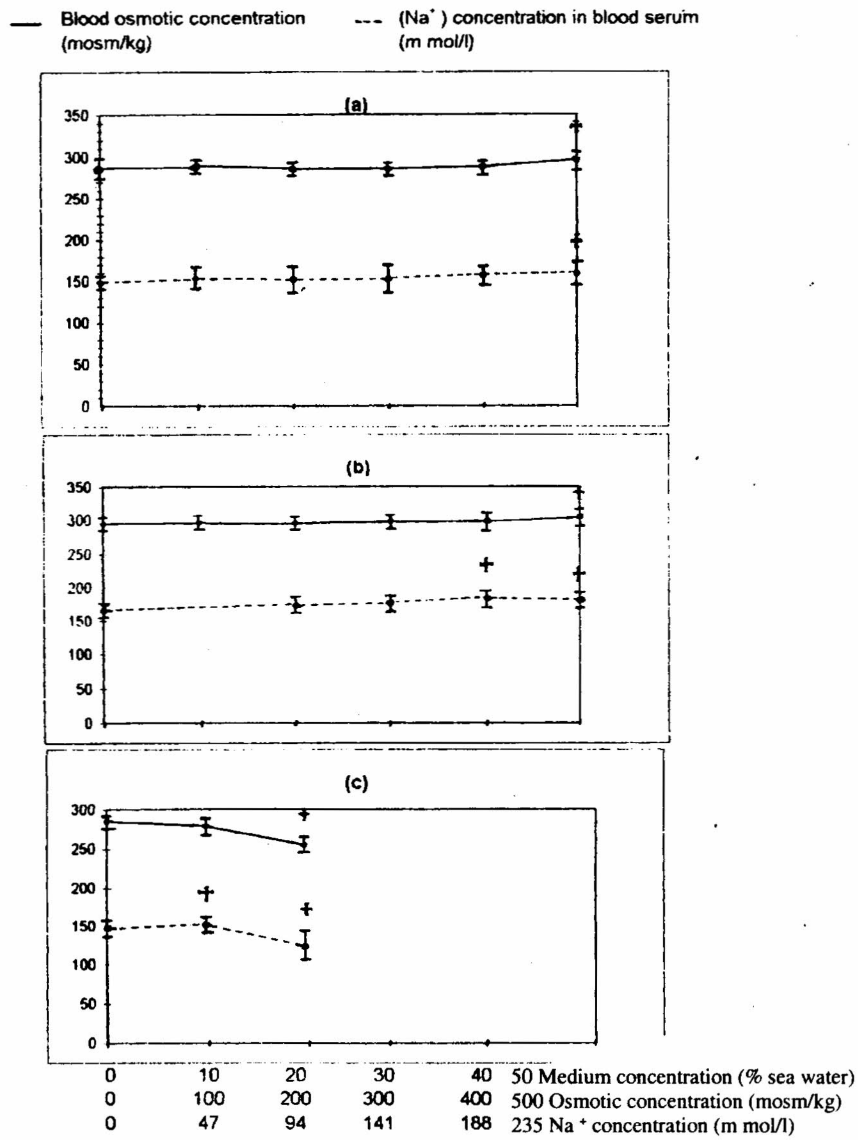


Figure 2 Effects of osmotic concentration of medium on osmotic concentration (-) and sodium concentration (---) of the blood serum of (a) *E-maculatus* (b) *E. suratensis* and (c) *Pantius filamentosus* (mean ± s. e. n-5) + indicate mean values significantly different ($p < 0.05$) from the fresh water values

Fig 2 a shows that in *E. maculatus* plasma osmotic concentrations and sodium concentrations were maintained at the fresh water level in fish acclimated to various salinities up to 40% sea water, the incipient lethal salinity for this fish. However, in 50% sea water significant increases in both osmotic and sodium concentrations were observed.

In *E. suratensis* plasma osmotic concentrations were maintained at the fresh water level in various salinities up to 40% sea water, the incipient lethal salinity. But at this salinity a significant increase in sodium concentration was observed (Fig2b). In 50% sea water both these parameters were significantly above the fresh water levels.

Fig 2c shows that for *P. filamentosus* plasma sodium concentration increased significantly in 10% sea water. However, both osmotic and sodium concentrations showed significant increases at the incipient lethal salinity of 20% sea water.

Energy expenditure

E.maculatus showed a significant reduction in energy expenditure at 30% sea water (Fig 3a).However, *E. suratensis* and *P.filamentosus* showed no significant changes in energy expenditure at salinities below their respective lethal levels (Fig 3b&3c).

Salinity preference

Significant increases in relative percentage frequency were observed for *E. maculatus* at 10%,20% and 40% sea water ($p<0.05$). But the highest significant values were recorded at ($p<0.01$) 10% and 20% sea water levels. A significant reduction in relative percentage frequency was observed at 50% sea water ($p<0.01$).

For *E.suratensis* the relative percentage frequency values were significantly increased (p.0.05) at 10% and 20% sea water. They also showed a significant reduction in relative percentage frequency at 40% and 50% sea water($p<0.01$).

P.filamentosus showed highest values in relative percentage frequency at fresh water ($p<0.01$) (Fig.4).

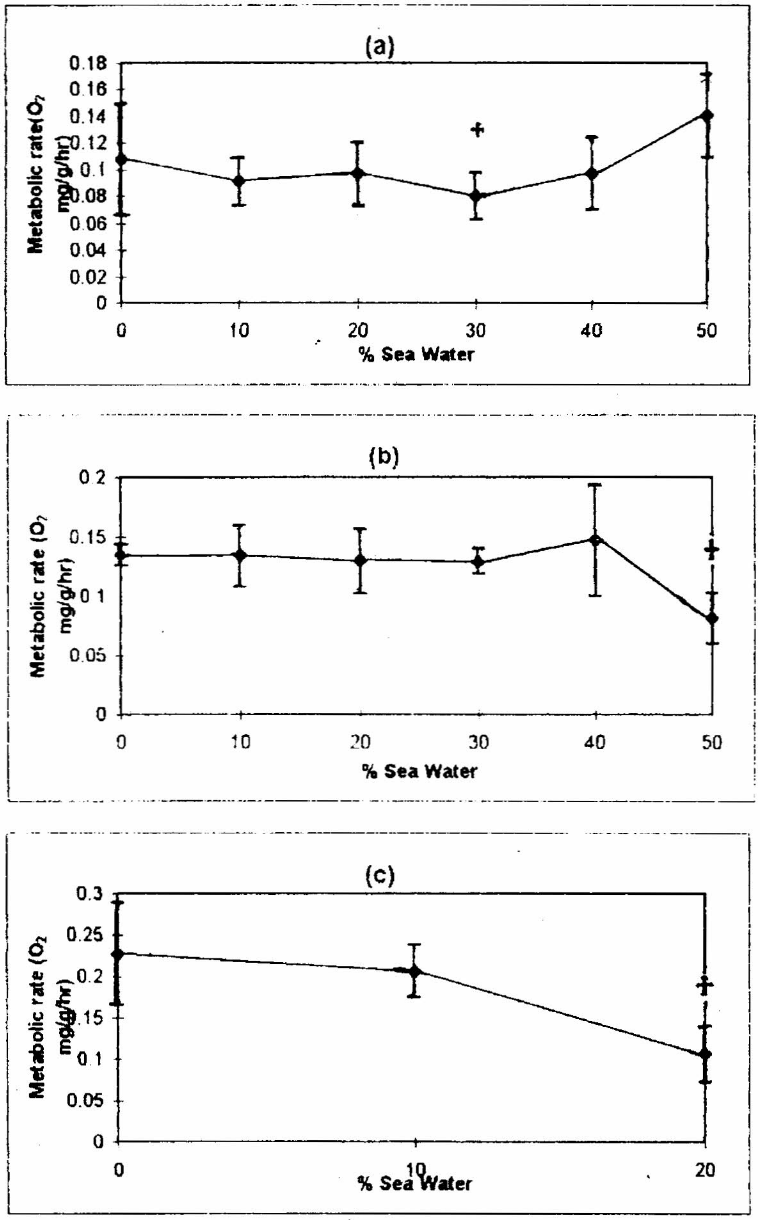


Figure 3 Meatbolic rate of *(a) Etroplus maculatus (b) E. suratensis (c)Puntius filamentosus* at different salinity media (mean ± s. e., -n=5)

+ indicate mean values significantly different ($p<0.05$) from the fresh water values.

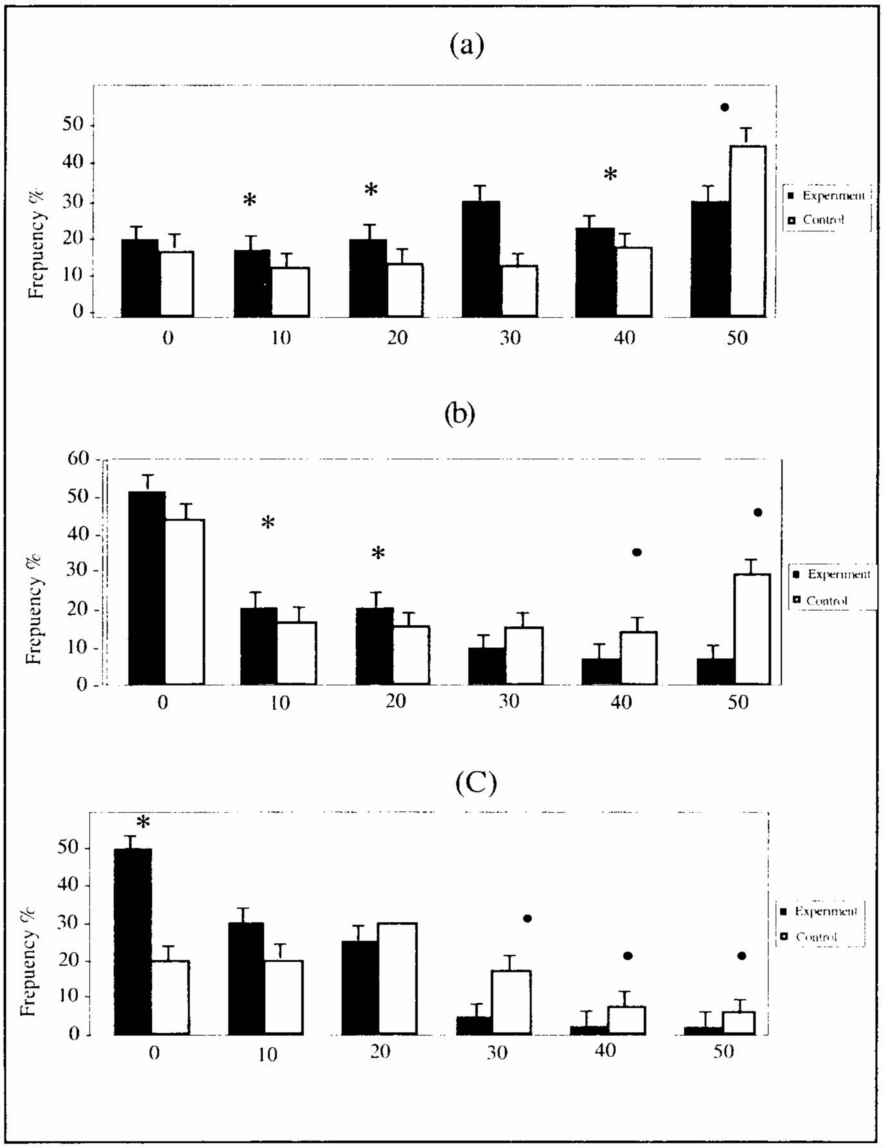


Figure 4 Frequency distribution of (a) *Etroplus maculatus* (b) *E. suratensis* (c) *Puntius filamentosus* in the experimental and control chambers. mean ± s.e. (N=8, n=384)

* Represent significant preferences

• Represent significant avoidances

N=number of fish, n=number of observations

Discussion

The tests for survival of *Etroplus maculatus* and *Etroplus suratensis* show that their salinity tolerances are considerably higher when compared to the salinity tolerance of *Puntius filamentosus*. *E.maculatus* and *E.suratensis* could survive in media up to 60% sea water (20.7ppt) after gradual acclimatization. 100% survival of fishes could be observed in salinities up to 30% sea water (10.35ppt). But *Puntius filamentosus* could survive in media only up to 20% sea water (6.9ppt) with gradual acclimatization.

Plasma osmotic concentrations and sodium concentrations recorded for *E. maculatus* and *E.suratensis* acclimated to media from fresh water to 40% sea water were maintained at constant levels. This reveals that both these species are good osmoregulators throughout this salinity range. But at salinities higher than 40% sea water significant increases in plasma osmotic concentrations and sodium concentrations were recorded for both species. For *P. filamentosus* at 20% sea water significant reductions of plasma osmotic concentration and sodium concentration were recorded. It can be concluded from these results that all these species lose their regulatory abilities when they reach their salinity tolerance limits. The breakdown of regulatory mechanisms permitting fatal changes in internal concentrations may be one reason for the deaths recorded for these species at their respective lethal salinity levels.

In *E.maculatus, E.suratensis* and *P.filamentosus* the sodium content recorded in the blood serum accounted for nearly 50% of the osmotic concentration. Thus sodium salts account for most of the osmotic concentration of the blood. Potts & Parry (1963) has shown that apart from the major osmotic constituent sodium salt, there are divalent ions such as Ca^{2+} and Mg^{2+} which account in some degree to the total osmotic concentration of the extracellular fluid of marine forms. The high contribution of sodium salt to the total osmotic concentration of the blood plasma of the experimental fish species is indicative of an evolution in fresh water.

When considering salinity tolerances, it was evident that there are clear cut differences between *Etroplus* spp and *P. filamentosus*. The high level of salinity tolerance ability of *Etroplus* spp may be due to the fact that these species belong to the order Cichlidae, while *P. filamentosus* belong to the order Cyprinidae. Cyprinids are primary fresh water fishes, while Cichlids are secondary fresh water fishes. Primary fresh water fishes are those that evolved purely in fresh water and secondary fresh water fishes are those that entered sea in their evolutionary history and returned back to fresh water. Thus secondary fresh water fishes have evolved mechanisms to tolerate higher salinities than the primary fresh water fishes . *Etroplus* spp are thus capable of tolerating higher salinities when compared to the primary fresh water fish *P. filamentosus*.

Most fresh water fishes face the problem of preventing dilution of body fluids by both osmotic influx of water and diffusional loss of salt. To reduce this problem they have mechanisms for active uptake of salts from the outside medium and they have well developed excretory organs to eliminate the excess water which enter by osmosis. Rankin and Davenport (1981) report that goldfish which had been kept in distilled water for some weeks were able to accumulate sodium chloride actively from solutions containing only 0.05M sodium chloride. Since all these activities require energy it may be related to the oxygen consumption and thereby to the metabolic rate of the animal. The relationship between the metabolic rate of the animal and active ion transport has been studied in many aquatic animals.In some cases a correlation has been found between the rates of ion transport and metabolic rate.Experiments carried out on Stickleback, *Gasterosteus* have shown that although it lives for long periods in fresh water it has a higher respiratory rate in fresh water than in isosmotic sea water (Potts & Parry,1963). The metabolic rate of hybrid *Tilapia, Oreochromis mossambicus* was lowest in isosmotic water (Potts & Parry,1963). A number of studies on other fish species have also indicated that their oxygen consumption is minimal near isosmotic salinity. But in many of the most competent regulators their respiratory rates are not affected by changes in salinity. Brackish water *Fundulus heteroclitus* respirates at the same rate both in fresh water and brackish water. Alevins of rainbow trout, *Salmo gairdnerii* and salmon, *Salmo salar* also show a similar pattern (Potts & Parry,1963).

The present study reveals that *E. maculatus* reduces its metabolic rate at its isosmotic salinity (30%sea water). But for *E.suratensis* no significant change in metabolic rate was recorded in the salinity range from fresh water to 40% sea water. This may be due to the fact that the energy spent for osmotic work by the animal is not large enough to represent the metabolic rate change or there may be a hormonal influence in salinity adaptation of this fish species influencing its basic metabolic pattern as in many euryhaline migratory fishes. But further investigations are necessary to conclude whether there is a hormonal influence in this species regarding their salinity adaptation. For *Puntius filamentosus* it was not possible to determine the metabolic rate at its isosmotic salinity (30% sea water) because it could not survive in 30% sea water. But no significant change in metabolic rate was obsrved at 10% sea water. Of the three species the highest metabolic rate was observed for *P.filamentosus* in fresh water. *E suratensis* and *P.filamentosus* had considerably reduced metabolic rates when they reached their upper salinity tolerance limits, since this level was lethal to the majority of fish.

The salinity preference tests indicate that *E.maculatus* and *E.suratensis* prefer salinities ranging from 10% sea water(3.45ppt) to 20% sea water (6.9ppt). *P.filamentosus* prefers fresh water.All the species evidently avoid higher salinities which may be because of the adverse effects on their physiological status at these salinities.

According to this experiment when the fresh water fishes were exposed for long periods to higher salinities loss of appetite was evident which is an indication of imbalance in physioligical mechanisms of the animals. The reduced appetite may also help them to avoid dietary intake of salts. A similar change in feeding behaviour accompaned by loss of body weight and mortality was observed in fresh water air breathing fish *Channa puntatus* when they were exposed to sodium chloride stress (Dheer *et al,* 1986). It was found that strees conditions induce depletion of energy reserves such as blood glucose level, muscle and liver glycogen content in those animals and stress symptoms in haematological parameters reflect as anaemia. It has been sugested that sodium chloride stress could lead to retardation in growth and loss of body weight.Similar changes can be expected in the experimental fish groups when they are exposed to higher salinities and this may be the reason for their preference for low salinity levels. When the extent of stress or its duration has crossed a particular limit, the lethal level, death would result.

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