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OSMOREGULATORY ADJUSTMENTS BY THREE ATHERINIDS (*LEPTATHERINA PRESBYTEROIDES*; *CRATEROCEPHALUS MUGILOIDES*; *LEPTATHERINA WALLACEI*) TO A RANGE OF SALINITIES

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Abstract—1. Patterns of osmoregulation were studied in three species of Swan river atherinids (*Leptatherina presbyteroides*, lower estuarine and marine; *Craterocephalus mugiloides*, mid estuarine; *Leptatherina wallacei*, upper estuarine) over a wide range of salinities.

2. The plasma Na^+ concentration was elevated with an increase in salinity.

3. Haematocrit and body water content decreased with acclimation to higher salinity.

4. All three species of atherinids osmotically regulated over a salinity range greater than that which these fish are reported to occur in.

INTRODUCTION

A number of atherinid fish (hardyheads) are found in the Swan–Avon river system, Western Australia, with different species having a preference for particular parts of the estuary (Prince *et al.*, 1982b). *Leptatherina presbyteroides* are found predominantly in the sea and lower estuary and to a lesser extent in the middle estuary. *Atherinosoma elongata* and *Craterocephalus mugiloides* are found at highest numbers in the middle estuary. In contrast, *Leptatherina wallacei* is found mostly in the upper and middle estuary, but is rarely caught in the lower estuary and is not found in the sea.

Diet and feeding patterns account for some of the spatial partitioning by these four atherinid species in the Swan estuary (Prince *et al.*, 1982b). The distribution of these fish changes seasonally depending on the salinity, but all species are known to occur at salinities higher than are found in the Swan river. However, it is not known if *L. presbyteroides* can survive in salinities lower than where it is normally found.

The purpose of this study was to determine the water and ion regulation patterns of these three hardyheads to a salinity range greater than that normally found in the Swan river estuary.

MATERIALS AND METHODS

Taxonomy and identification of atherinids followed Pavlov *et al.* (1988) for *L. presbyteroides* and *L. wallacei* and Crowley and Ivantsoff (1988) for *C. mugiloides*. These three hardyheads are similar morphologically and considerable practice was required to reliably identify species in the field.

Salinity measurement

A Yeo-Kal temperature–salinity bridge (model 602) was used to measure salinity of water below 62 g/l in the field and laboratory, and a refractometer (Scientific Instruments, Series # cat. 10419) was used to measure salinity levels greater than 62 g/l in the laboratory; values were read to ±1.0 ppt and reported as g/l.

Fish collection and maintenance

Adult *L. wallacei* and *C. mugiloides* were collected in October–November 1989 from the Swan river using a seine net. The salinity at the collection sites for *L. wallacei* and *C. mugiloides* was 5 and 11 g/l for the respective species; surface water temperature was 19°C. The fish were transported to the laboratory and maintained in aquaria at a salinity of 5 and 11 g/l, respectively, and a temperature between 19 and 21°C for a minimum of 4 days. *L. wallacei* were then randomly divided into five groups, and the salinity of the water in each aquarium was adjusted over a period of 7 days, by adding rain water or sodium chloride, to 4–86 g/l. *C. mugiloides* were randomly divided into four groups and similarly acclimated to 6–60 g/l over 7 days. These salinity ranges reflect the widest known range reported for both species. The salinity remained unchanged in the aquariums over the 7 days prior to plasma Na^+, haematocrit and body water content being determined.

L. presbyteroides were collected in February 1990 near the mouth of the Swan river at a salinity of 34 g/l. Earlier attempts to capture and transport these fish to the laboratory resulted in the death of almost all individuals, as was reported by Prince *et al.* (1982a) but it was found that capturing the fish at night resulted in minimal deaths. These fish were maintained in aquaria in the laboratory at a salinity of 35 g/l and 17–19°C for 7 days before being divided into three groups, with salinity being progressively adjusted from 5–71 g/l over 5 days. These adjusted salinity levels were maintained until blood samples were taken and body water content measured after a further 9 days.

All fish were housed in aquaria, and were fed commercially available fish food preparations, grated beef heart and fish. Minimal mortality was experienced after 5 days when it appeared that fish had adjusted to the aquarium environment.

Blood sampling

Fish were removed from their aquarium, scales cleaned away from the area anterior to the ventricle and the skin was cut to expose the heart. A 20 μl heparinized capillary tube (Drummond Microcaps) was used to puncture the ventricle

and extract blood. The fish were then immediately decapitated. The blood was centrifuged for 3 min and the volume of red blood cells was then measured as a percentage.

One microlitre of plasma was placed in 3 ml of CsCl diluent for analysis of Na^+ concentration using a Varian 475 Atomic Absorption Spectrophotometer.

Body water content

L. wallacei and *L. presbyteroides* were removed from their aquarium, dried with absorbent tissue and immediately decapitated. The fish were then cut into three pieces and weighed. The fish were then dried in an oven at 55°C for a period of 7 days before being reweighed. The percentage of dry body wt to total body wt was calculated. No further change in body water content was evident after a further 3 days.

The mean and SE for Na^+, haematocrit and percentage body water content at differing acclimation salinities were calculated and analysed for each species by one way analysis of variance; where a difference exists Tukey's Multiple Comparison procedure was used to determine the difference between means (Kitchens, 1987). Interspecies variations were tested by analysis of covariance, using all data for each species at each salinity (Zar, 1984).

RESULTS

There is a significant increase ($F_{2,36} = 56.1$; $P < 0.01$) in plasma Na^+ concentration with elevated salinity for *L. presbyteroides* with the mean values being 149.7 mM at 5 g/l; 178.3 mM at 37 g/l; and 249.6 mM at 71 g/l. There is a decrease ($F_{2,42} = 12.9$; $P < 0.01$) in body water content with elevated salinity with the mean values being 75.1% at 5 g/l; 72.2% at 37 g/l; and 71.4% at 71 g/l for *L. presbyteroides*.

Haematocrit significantly decreased ($F_{2,31} = 11.2$; $P < 0.01$) with increasing salinity for *L. presbyteroides* with the mean values being 38.8% at 5 g/l; 29.7% at 37 g/l; and 27.8% at 71 g/l (Fig. 1).

Plasma concentration of Na^+ increased ($F_{3,25} = 15.9$; $P < 0.01$) for *C. mugiloides* with the mean values being 146.5 mM at 6 g/l; 187.6 mM at 38 g/l; 203.6 mM at 56 g/l; and 214.7 at 60 g/l. Haematocrit decreased ($F_{3,24} = 5.9$; $P < 0.01$) with increasing salinity for *C. mugiloides* with the mean values being 31.6% at 6 g/l; 30.4% at 38 g/l; 25.7% at 56 g/l; and 22.9% at 60 g/l (Fig. 1).

L. wallacei plasma Na^+ concentration increased ($F_{4,39} = 31.6$; $P < 0.01$) with elevated salinity with the mean values being 153.8 mM at 4 g/l; 163.6 mM at 12 g/l; 190.6 mM at 36 g/l; 200.8 mM at 50 g/l; and 243.6 mM at 86 g/l. Body water content significantly decreased as increasing salinity ($F_{2,42} = 21.9$; $P < 0.01$) for *L. wallacei* with the mean values being 78.5% at 4 g/l; 77.7% at 6 g/l; 77.1% at 37 g/l; and 76.3% at 56 g/l. *L. wallacei* haematocrit decreased ($F_{4,43} = 21.5$; $P < 0.01$) with elevated salinity with the mean values being 36.1% at 4 g/l; 38.4% at 12 g/l; 27.7% at 36 g/l; 26.3% at 50 g/l; and 19.8% at 86 g/l (Fig. 1).

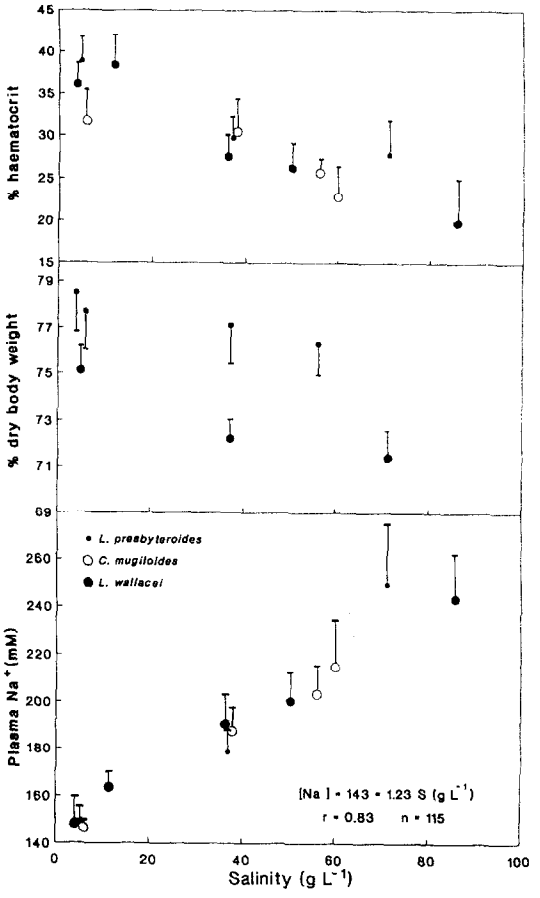

Fig. 1. Haematocrits, dry body wt as a percentage of wet wt and plasma sodium concentrations for *L. presbyteroides*, *C. mugiloides* and *L. wallacei* at varying salinities. Values are the means and two standard error bars (shown only in one direction for clarity).

DISCUSSION

Teleost fish normally maintain a "steady state" of their body fluid ion concentrations and intracellular and extracellular fluid volumes at a constant salinity. There is a range of salinity tolerance, below and above which iono- and osmoregulation fail to maintain homoeostasis and the fish die, but the tolerance range varies markedly for different fish. In fresh water, fish are hyper-osmotic to the medium and tend to gain water by osmosis and ingestion and lose solutes by diffusion and excretion. Marine teleosts are hypo-osmotic to sea water and lose water osmotically and by excretion and gain solutes by diffusion and ingestion.

Tolerance to salinity

All three atherinids tolerated salinity levels in excess of that found in any section of the Swan–Avon river estuary for a minimum period of 7 days. *L. wallacei* tolerated salinity levels up to at least 85 g/l, *L. presbyteroides* to at least 71 g/l and *C. mugiloides* up to 60 g/l. Their presence in specific parts of the Swan river therefore cannot be attributed simply to short-term salinity tolerance.

Atherinids became noticeably emaciated in the abdominal region at higher salinities, and this may have been the cause of death. This emaciation may be due to the fish not eating, to restricting the intake of ions by ingestion, or by the depletion of body energy stores due to the increased metabolic cost associated with maintaining osmotic balance in hyperosmotic media.

Plasma Na^+ *concentration*

Plasma Na^+ concentrations have been reported for numerous teleosts over the last 30 years; see reviews of Holmes and Donaldson (1969) and Evans (1979). It can be concluded from these reviews that the plasma concentration of Na^+ increases with elevated salinity for euryhaline teleosts moved between fresh and sea water. Whether this relationship continues continues beyond sea water salinity levels for euryhaline fish that frequent hypersaline environments such as salt lakes has seldom been examined (House, 1963). Nordlie and Walsh (1989) found an approximately linear relationship between plasma osmotic concentration and salinity for three cyprinodontid teleosts between fresh water and sea water but a disproportionate increase in plasma Na^+ at higher salinities.

The plasma Na^+ concentrations for *L. presbyteroides*, *C. mugiloides* and *L. wallacei* acclimated to very dilute sea water and sea water are equivalent to those reported for other euryhaline teleosts and summarized by Holmes and Donaldson (1969). Assuming a linear relationship between plasma Na^+ concentrations and acclimated salinity levels, an ANCOVA revealed no significant difference between species ($P > 0.05$ for elevation and slope) over the salinity ranges tested. The regression equation Na^+ (mM) = 143 + 1.23 salinity ($r = 0.83$) represents the relationship between plasma Na^+ conentration and salinity based on the combined data for the three hardyheads. This apparent linear relationship appears to extend to salinity levels well beyond that of sea water, and up to at least 60 g/l for *C. mugiloides*, 71 g/l for *L. presbyteroides* and 85 g/l for *L. wallacei*.

Body water content

Salmonids moving from a fresh water environment to the sea are able to maintain a fairly constant total body water content (Parry, 1966). Lange and Fugelli (1965) report a small increase in body water content for *Pleuronectes flesus* and *Gasterosteus aculeatus* when transferred from sea water to fresh water. Similar findings are reported by Parry (1961) and Gordon (1959). Black (1948) reported that *Fundulus heteroclitus* had an increased body fluid content soon after being transferred from sea water to fresh water (an increase in 6.6% of total body water in 7 hr), followed by a decrease to the initial level after about 15 hr, and a further rapid decrease for the rest of the first 24 hr. The process was reversed when fish were transferred from fresh water to sea water. Ahokas and Duerr (1975) reported an increase in body water content for *Culaea inconstanus* when acclimated from fresh water to 17.5 g/l whereas the trend was less obvious in *Fundulus diaphanus* when transferred from fresh water to a hypersaline medium (40 g/l).

There was a significant reduction in the body water content of *L. presbyteroides* and *L. wallacei* at high salinities. This is presumably a consequence of the greater osmotic pressure gradient that promotes water loss from the fish to the hypersaline medium. To test for significant differences in water content between the species, we assumed linearity and used ANCOVA. Based on this analysis, there is a significant difference between the slopes ($P < 0.05$) and elevations ($P < 0.01$) for the regression equations relating dry body wt to salinity levels for *L. presbyteroides* and *L. wallacei*. The relatively higher body water content of the low-salinity *L. wallacei* compared with the higher-salinity *L. presbyteroides* is not necessarily as an osmoregulatory adaptation, as the body water content for each species is within the range expected for euryhaline teleosts.

Haematocrit

Haematocrits have been reported for many teleosts; there is a considerable variation between and within species. There appears to be no information on changes in haematocrit levels in response to changes in salinity from fresh water to hypersaline levels.

The haematocrits of atherinids acclimated to salinity levels between fresh and sea water are within the range reported for other teleosts see Rakitskaya (1982) and reviews by Holmes and Donaldson (1969) and Moyle and Cech (1988). Haematocrits of the three atherinid species significantly decrease ($P < 0.01$) as salinity levels increase from fresh to sea water. There is no significant difference between species (ANCOVA, $P > 0.05$ for elevation and slope). Haematocrit declined further at salinity levels higher than sea water. The relationship between haematocrit and salinity appears fairly linear over the range tested and it is represented by the following regression equation; haematocrit = 37.2 − 0.195 salinity ($r = 0.69$). It is not known whether this decrease in haematocrit is a result of a change in the red blood cell numbers or volume or a change in the plasma volume, but it does occur despite a decline in body water content.

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