Osmoregulation in three species of Ambassidae (Osteichthyes: Perciformes) from estuaries in Natal

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Received 25 July 1989; accepted 17 July 1990

Whole blood osmotic regulation was evaluated in three co-occurring, estuarine species of *Ambassis* exposed to ambient salinities from fresh water (0,13‰) to 53‰. Blood molality of all three species, acclimated to fresh water, showed significant increases over the range of ambient salinities from fresh water through 5‰ and stabilized only in the range 18‰–35‰). Osmotic concentrations of all three species rose abruptly at salinities above 35‰ and no species survived direct transfer into ambient salinities above 53‰. *A. productus*, collected in fresh water, required 24 h prior acclimation at 18‰ for survival in sea water (35‰). *A. gymnocephalus* acclimated in sea water showed the least tolerance of the three species to low salinities and experienced a 42% decrease in blood osmotic concentration when exposed to fresh water whereas the decrease for *A. natalensis* and *A. productus* was only 20% and 23% respectively. Histological investigation of *Ambassis* kidneys indicated that all three species have structurally advanced kidneys of the mesonephric type common to most teleosts which spend a proportion of their lives in a hyposmotic medium. Osmotic regulatory characteristics of *Ambassis* species are discussed in relation to their distribution in estuaries.

Die regulering van bloedosmose van die drie *Ambassis*-spesies wat in riviermondings voorkom en blootgestel is aan omringende water met soutgehaltes vanaf varswater (0,13‰) tot 53‰ is ge-evalueer. Die bloedmolaliteit van al drie spesies wat in varswater aangepas was, het beduidende toenames getoon in soutgehaltes vanaf varswater tot 5‰ en het slegs in die bestek 18‰–35‰ gestabiliseer. Die osmotiese konsentrasie van al drie spesies het by soutgehaltes bo 35‰ skielik gestyg en geen spesie het 'n direkte oorplasing na 'n omringende soutgehalte van meer as 53‰ oorleef nie. *A. productus*, wat in varswater versamel is, moes eers vir 24 h in 18‰ soutwater aangepas word voordat dit in seewater (35‰) kon oorleef. Na aanpassing in seewater het *A. gymnocephalus* die minste verdraagsaamheid teenoor 'n lae soutgehalte getoon en 'n 42% afname in die osmotiese konsentrasie van bloed na blootstelling aan varswater. In teenstelling hiermee is 'n afname van slegs 20% en 23% onderskeidelik, by *A. natalensis* en *A. productus* aangetref. Histologiese ondersoeke van *Ambassis*-niere het aangedui dat al drie spesies struktureel gevorderde niere van die mesonefriese tipe het. Hierdie niere kom algemeen voor in die meeste beenvisse wat 'n gedeelte van hulle lewe in 'n hyposmotiese medium deurbring. Osmoregulerende eienskappe van *Ambassis*-spesies met betrekking tot hulle verspreiding in riviermondings word bespreek.

Ambassidae are represented in South African estuaries by three species which are very similar in external morphology (Martin & Heemstra 1988), feeding ecology (Martin & Blaber 1983), alimentary system (Martin & Blaber 1984) and distribution (Martin 1983). The co-existence and spatial separation of these species of *Ambassis* in the estuaries of southern Africa can be explained by the tolerance of each to salinity and temperature and their specific ability to osmoregulate under estuarine conditions. Investigations of the temperature tolerance ranges of the three species (Martin 1988) suggest that the survival capability of *Ambassis productus* in reduced salinities (< 10‰) increases while that of *A. gymnocephalus* decreases sharply in salinities below 20‰. *A. natalensis*, which is endemic to the south-east coast of Africa, is adapted to a wide range of estuarine conditions. Interaction between salinity and temperature on the tolerance limits of *Ambassis* spp. is significant with regard to the occurrence and spatial separation of the three species in estuaries (Martin 1988).

The aim of the present work was to study the osmoregulatory capabilities of the three species and relate this to previous work on their temperature tolerances and distribution in estuaries (Martin 1988).

Materials and Methods

Fish

Adult *A. natalensis* (45–55 mm SL) and *A. gymnocephalus* (40–50 mm SL) were collected from estuaries along the Natal coast (South Africa) by seine net and transported in estuary water to the laboratory in 251 containers equipped with aerators. Fish were acclimated and maintained for 14 days at 25°C (±1,5°C) in 45 l glass aquaria at a stocking density of one fish per 3,75 l. Each aquarium contained synthetic seawater (34‰) and was fitted with air lift filtration. Fish were fed twice daily on a diet of commercial aquarium fish food until the day before experimentation. Experiments were performed in glass aquaria using 40 l of continuously aerated and filtered water. In each experiment fish were transferred directly from the acclimation tanks to the experimental aquaria. All experiments were run at 25°C (± 1,5°C). Experimental salinities were varied by dissolving different amounts of 'Synthetica Sea Salt' in tap water (3 mOsmol kg^{-1}). Salinities were measured by $AgNO_3$ titration. Owing to difficulties experienced in capturing and transporting *A. productus* over long distances to the laboratory, a temporary field station was set up at Lake Nhlange, Kosi Bay (26°47'S/32°47'E). Fish were netted in

the lake and acclimated for a minimum of 24 h in filtered lake water (0,30‰ or 5 mOsmol kg^{-1}) at 25°C (± 1,5°C) before being transferred directly into aquaria of different experimental salinities.

Experiments

The osmotic concentration of whole blood was measured in milliosmoles per kilogram (mOsmol kg^{-1}) using a Wescor 5100 Vapor Pressure Osmometer. Samples of five fish at a time were anaesthetized with Sandoz MS 222. Owing to the small size of *Ambassis* it was not possible to obtain sufficient whole blood (8µl) samples for use in the conventional methods recommended by the Wescor Osmometer manufacturers. Instead blood samples were obtained by sample disc saturation consistent with bulk-sampling methods described in the Wescor Osmometry Bulletin (1981). With the fish lying right side down, excess moisture was adsorbed from the inner and outer surfaces of the first gill arch using lint-free lens tissue. A Wescor sample disc was inserted between the first and second gill arch and the afferent branchial artery of the first gill arch was severed allowing the sample disc to become completely saturated with blood. The saturated disc was immediately transferred to the osmometer and the osmotic concentration of the whole blood was determined.

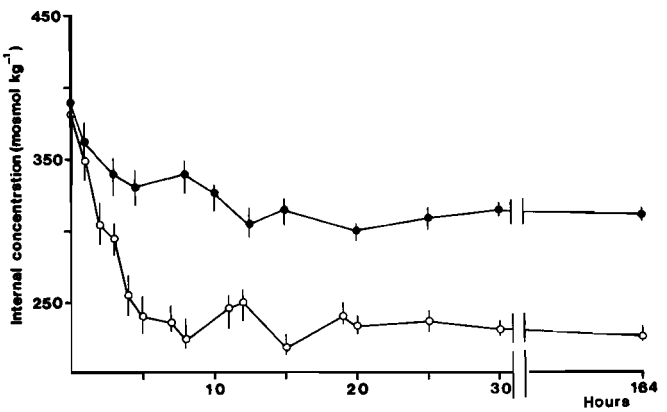

Figure 1 Changes in the osmotic concentration of the blood of *A. natalensis* (●) and *A. gymnocephalus* (○) over a period of time after transfer from 34,44‰ to freshwater. Actual range of variation shown for each point (mean of five fish).

Kidney structure

The internal structure of the kidneys of the three *Ambassis* species was investigated from prepared histological sections stained with haemotoxylin and eosin. Photomicrographs were taken to illustrate the significant features relevant to efficient osmoregulation in media hyposmotic to the blood.

Results

Osmoregulation

Figure 1 shows the time taken by *A. natalensis* and *A. gymnocephalus* to adjust osmotically to freshwater (3 mOsmol kg^{-1}) after acclimation in water of 978 mOsmol kg^{-1} (34,44‰) for 14 days. After a rapid decrease during the first 5 h the internal concentration stabilized within 20 h at a mean of 307 mOsmol kg^{-1} and 224 mOsmol kg^{-1} for *A. natalensis* and *A. gymnocephalus* respectively. The significant decrease in internal concentration (379–224 mOsmol kg^{-1}; $t = 30{,}862$; $p < 0{,}01$) shown by *A. gymnocephalus* initially suggested a high degree of osmoregulatory collapse owing to partial haemolysis. However, subsequent microscopical inspection of whole blood from these fish showed the erythrocytes to be intact.

Figure 2 shows the time taken for *A. productus* and *A. natalensis* to adjust internally to experimental salinities after direct transfer from freshwater (0,3‰). *A. productus* was unable to survive direct transfer into seawater of 36‰ (1025 mOsmol kg^{-1}) and 100% mortality occurred after 4 h. They did, however, withstand direct transfer into diluted seawater of 18 and 25‰. Blood osmolality increased rapidly during the first hour and then decreased to a stable mean of 322 mOsmol kg^{-1} ($p < 0{,}05$) after 15 h for fish in both 18‰ and 25‰. Fish acclimated at 18‰ for 15 h were subsequently able to withstand full seawater and adjustment to a mean blood osmolality of 362 mOsmol kg^{-1} ($p < 0{,}01$) was complete after 7 h. *A. natalensis* tolerated direct transfer from

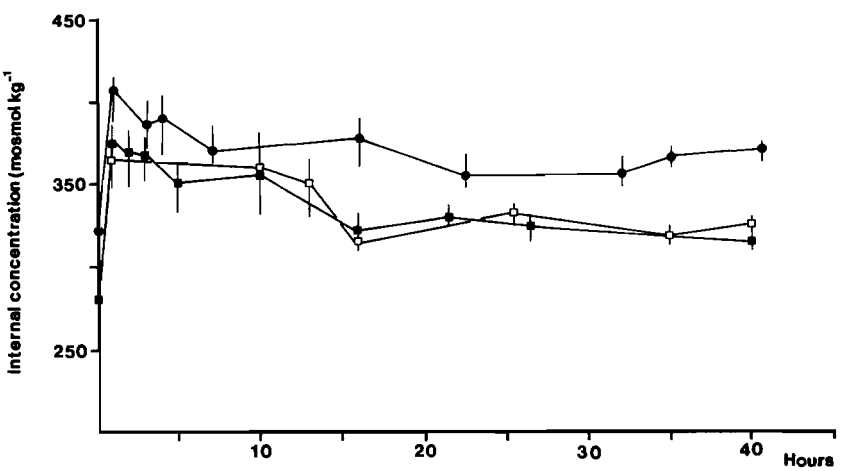

Figure 2 Changes in the osmotic concentration of the blood of two *Ambassis* spp. acclimated in freshwater and exposed to different ambient salinities over a period of time. Actual range of individual variation is shown for each data point (mean of five fish). (□) *A. productus* in 25‰; (■) *A. productus* in 18‰; (●) *A. natalensis* in 35‰.

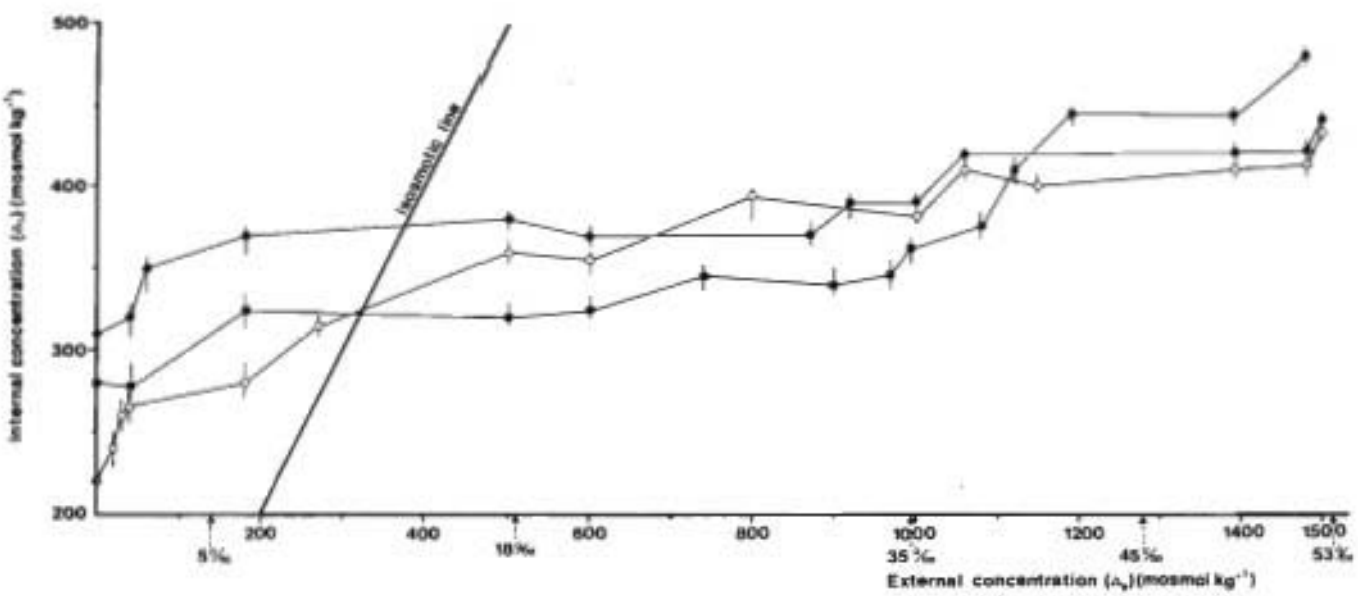

Figure 3 Osmotic concentration of the blood of Ambassidae after 20 h in a range of ambient salinities from freshwater to 53‰. Actual range of individual variation shown for each data point (mean of five fish). ■ *A. productus;* ● *A. natalensis;* ○ *A. gymnocephalus.*

fresh to seawater without mortality.

Figure 3 shows the osmotic concentration of the blood of all three *Ambassis* species after 20-h exposures in a range of experimental salinities from freshwater to 53‰. Generally internal concentrations of all three species, acclimated in freshwater, showed a marked increase over the range of ambient salinities from freshwater through 5‰. Thereafter internal concentrations increased more slowly and eventually stabilized in ambient salinities above 18‰. An upward trend began again only above 30‰ with marked increases at ambient salinities between 35‰ and 40‰. *A. productus* did not survive more than 20 h at 52‰ while *A. natalensis* and *A. gymnocephalus* tolerated 52‰ but died after 80 h at 53‰.

The decrease in blood osmotic concentration between 18‰ and freshwater was 141 mOsmol kg^{-1} for *A. gymnocephalus* (t = 38,66; $p < 0,01$), 69 mOsmol kg^{-1} for *A. natalensis* (t = 17,04; $p < 0,01$) and 42 mOsmol kg^{-1} for *A. productus* (t = 12,44; $p < 0,01$). These values represent a decrease of 39%, 18% and 13% over the level in an ambient medium of 18‰ for *A. gymnocephalus*, *A. natalensis* and *A. productus*, respectively. Over the ambient range 35‰ to 18‰, the decrease in internal concentration was considerably less for all three species and was calculated over the level in an ambient medium of 35‰ at 19 mOsmol kg^{-1} (5%) for *A. gymnocephalus* ($p < 0,05$); 9 mOsmol kg^{-1} (2,3%) for *A. natalensis* ($p < 0,05$), and 42 mOsmol kg^{-1} (11,6%) for *A. productus* ($p < 0,05$). The overall percentage decrease in blood osmotic concentration for each ambassid species over the ambient range from seawater (35‰) down to freshwater (0,3‰) was 42% for *A. gymnocephalus* ($p < 0,01$), 20% for *A. natalensis* ($p < 0,01$), and 23% for *A. productus* ($p < 0,01$).

Histology of the kidney

The kidneys of the three species are similar in structure and possess nephrons which resemble the euryhaline glomerular

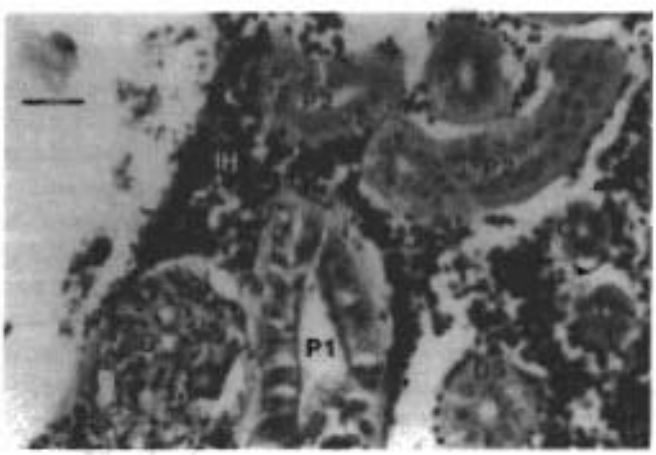

Figure 4 Transverse section of *A. natalensis* kidney. Scale bar = 2 µm. (C, collecting duct; DS, distal segment; G, glomerulus; IH, interstitial haemopoietic tissue; P1, first proximal segment; P2, second proximal segment.)

type described by Hickman & Trump (1969). Figure 4 shows the following regions: a well vascularized glomerulus, (G); first proximal segments, (P1); second proximal segments, (P2); collecting tubules, (C); interstitial haemopoietic tissue, (IH); and distal segments, (DS) lined by large clear cells. The segmented arrangement of the nephrons indicates that the kidney of Ambassidae is a structurally advanced filtration-resorption unit of the mesonephric type found in most teleosts which spend a proportion of their lives in a hyposmotic medium (Hickman & Trump 1969). For fish, a mesonephric type of kidney is able to function largely as a water excretory device in a hyposmotic medium or freshwater, but in salt water, hyperosmotic to the blood, the kidney functions chiefly as an excretory device for magnesium and sulphate ions and the conservation of water and monovalent ions (Hickman & Trump 1969; Bone & Marshall 1982).

Discussion

The responses of *A. natalensis* and *A. gymnocephalus* to reduced salinities (Figure 1) are reflected by their blood osmolality. The rapid drop in whole blood osmolality during the first 5 h after transfer from seawater into freshwater suggests that *Ambassis* are able to tolerate rapid salinity changes by lowering their blood osmolality and thus reducing the osmotic gradient between the internal and external media. Rapid adjustment over a short time period when transferred from seawater into freshwater is characteristic of other euryhaline species such as *Pleuronectes flesus* (House 1963), *Alosa sapidissima* (Leggett & O'Boyle 1976) and *Mugil cephalus* (Nordlie, Szelistowski & Nordlie 1982).

There is evidence that marine species which frequent estuaries during their juvenile stages may have different responses from *Ambassis* species to reduced salinities. *Rhabdosargus holubi* does not respond to short-term changes in salinity and can maintain a high internal concentration for 10 h before lowering its blood osmolality in nearly freshwater (3,5‰) (Blaber 1974).

Adaptation after a change from freshwater to seawater is dependent on the period spent in freshwater (Holliday 1971; Nordlie 1985). After long-term adaptation to freshwater in Lake Nhlange, *A. productus* were unable to tolerate direct transfer into seawater and died within 6 h. Survival depended on a 24-h acclimation period in 18‰ prior to transfer into seawater (Figure 2). During this study, Mdloti Estuary (29°38'S/31°08'E) was closed to the sea for a period of several weeks and became fresh. *A. productus* captured in freshwater at Mdloti and placed directly into seawater for transport to the laboratory, survived without acclimation. This suggests that *A. productus* are capable of retaining their osmoregulatory capabilities for at least a few weeks after acclimation to freshwater. There remains a need therefore to evaluate more fully the retention of osmoregulatory capabilities of this species after acclimation to freshwater.

For *Ambassis* species distributed widely throughout closed and open estuaries in Natal, rapid adjustment to long or short-term changes in salinity can be advantageous, especially when salinities change rapidly during freshwater flooding or from tidal action. Reduction of the osmotic gradient between the body tissues and external media may conserve energy which can then be used more profitably for foraging and predator avoidance activities. Although published accounts of metabolic energy costs in various salinities are conflicting (Kinne 1971; Priede 1985), some workers agree that the metabolic costs of maintaining osmotic gradients are high for fish living in seawater (Job 1959) and freshwater (Canagaratnam 1959). Salinities least demanding energetically were found to be between 25% and 50% seawater for *Lebistes verticulatus* (Gibson & Hirst 1955) and 50% seawater for *Ambassis interrupta* (Nordlie 1978). In *Ambassis* species used in this study survival rates at high and low temperatures were greatest in concentrations of between 25% and 57% seawater (Martin 1988). Hickman (1959) found that the metabolic rates of *Platichthys stellatus* in salinities above 35‰ are significantly greater (approximately 15%) than in normal seawater. Some of the extremely euryhaline forms (*Mugil cephalus* and *Onchorhynchus mykiss*) allow their blood osmolality to vary considerably depending on ambient concentrations (Madan Mohar Roa 1968, 1971; Nordlie *et al.* 1982) whereas others with similar euryhaline abilities such as *Salmo salar* (Parry 1961) and *Gasterosteus aculeatus* (Koch & Heuts 1943) allow much less variation in their internal concentration.

Ambassis fall into an intermediate category. At salinities between 50% (18‰) and 100% (35‰) there is comparatively little variation in the whole blood osmolality of all three species (Figure 3) but at salinities above 35‰ the internal concentration rises rapidly before stabilizing at a higher level until lethal salinities (53‰) are reached (Figure 3). A similar response to salinities above a critical level was reported by Whitfield & Blaber (1974) for *Tilapia rendalli* inhabiting estuarine areas in Natal.

It is normal for euryhaline teleosts to undergo changes of 20 to 30% in blood osmotic pressure after transfer from seawater into freshwater (Huggins & Colley 1971). Although laboratory tests have shown that *A. gymnocephalus* can survive extended periods (21 days) in freshwater (Martin 1988), the large change (42%) in blood osmolality after transfer from seawater into freshwater (Figure 3) suggests that this is the least euryhaline of the three species. Although a change of this magnitude suggests near osmoregulatory collapse for this species, it is characteristic of some stenohaline species which enter estuaries (Blaber 1974). Blaber (1974) found that the concentration of the blood of juvenile *R. holubi* changed from 370 mOsmol kg^{-1} in seawater to 216 mOsmol kg^{-1} in water of 1‰. This represents a change of 41% which is similar to the 42% change recorded for *A. gymnocephalus*. It is noteworthy that the per cent change in blood osmolality (28%) for *T. rendalli*, calculated from the results obtained by Whitfield & Blaber (1974), is within the range quoted for euryhaline teleosts, although this species is essentially freshwater and cannot tolerate salinities in excess of 19‰.

Gunter (1956) defined a euryhaline fish as 'one which has been recorded from both freshwater and seawater by competent observers'. This definition includes anadromous and catadromous forms but excludes those species that enter bays of low salinities. Since *A. gymnocephalus* has never been recorded in freshwater in Natal it is by Gunter's definition a stenohaline species. The inability to maintain a stable internal concentration in a hyposmotic medium (< 10‰) (Figure 3) is clearly a contributing factor which in Natal restricts *A. gymnocephalus* to the mouths of permanently open estuaries where access to inshore marine areas is possible during times of lowered salinity within an estuary. The distribution of *A. gymnocephalus* in the rest of the Indo-Pacific also appears to be marine. Natarajan & Patnaik (1968) and Chua (1973) describe this species as being abundant over the shallow bars separating most shallow marine areas and coastal lakes from the sea in India and south-east Asia. Haines (1979) recorded *A. gymnocephalus* as abundant in the Purari River mouth (New Guinea). In a recent survey of fish on the north-west coast of Australia, S.J.M. Blaber (pers. comm.) recorded *A. gymnocephalus* as abundant along the shore in shallow marine conditions.

The changes in internal osmotic pressure of 23% for *A. productus* and 20% for *A. natalensis*, when transferred from seawater to freshwater, are within the limits (20 to 30%) quoted for euryhaline teleosts by Gilles (1975). This

suggests that both are efficient osmoregulators in freshwater and seawater. In salinities greater than seawater (35‰) *A. natalensis* and *A. gymnocephalus* are able, after a slight increase in blood osmolality, to maintain a stable internal concentration in salinities up to 52‰ (Figure 3). However, for *A. productus* exposed to ambient salinities above 35‰, the internal concentration rises steadily, resulting in a significantly higher final blood osmolality (478 mOsmol kg^{-1}) than either of the other two species. *A. productus* is thus an efficient osmoregulator in salinities below 35‰ but is unable to maintain its internal concentration as efficiently as the other two *Ambassis* species in salinities above seawater. The inability to osmoregulate efficiently in salinities above 35‰ may be one of the factors restricting *A. productus* mainly to low salinity areas (< 10‰) within the estuaries of Natal (Martin 1988). In St Lucia (28°23'S/ 32°26'E) where salinities frequently exceeded 35‰ and low salinity areas were scarce, *A. productus* numbers were exceptionally low compared with the other two species (Martin 1983).

The function of the teleost kidney in regulating the internal concentration of body fluids has been well documented by Grasse (1953), Hickman & Trump (1969) and Gilles (1975). The presence of several freshwater aglomerular syngnathids (Grasse 1953) are evidence that the glomerulus is not an essential device for life in hyposmotic media. It is also apparent that while glomeruli occur mostly in freshwater fishes they are also an important preadaptation to marine and freshwater species entering estuaries (Gilles 1975) and in *Ambassis* spp. probably help to maintain the internal concentration hyperosmotic in low salinities (< 350 mOsmol kg^{-1}) and hyposmotic in higher salinities (> 400 mOsmol kg^{-1}) (Figure 3). Most marine teleosts have glomerular kidneys (Hickman & Trump 1969) and according to Gunter (1956) most euryhaline species are of marine origin. This suggests that the kidney of marine fishes has a wider adaptive capability than the kidney of freshwater fishes. Thus Ambassidae, possessing strong marine affinities and glomerular kidneys (Figure 4) are adapted to live in estuaries where salinity can vary from almost freshwater to full seawater in a matter of hours. The structure of the Ambassidae kidneys (Figure 4) with nephrons of the euryhaline marine type (Hickman & Trump 1969) and well-developed glomeruli helps explain firstly, how Ambassidae are able to osmoregulate for extended periods in freshwater and secondly, their tolerance to changes in salinity over a wide range and lastly their distribution in both closed and open estuaries in Natal.

Acknowledgements

This work was financed by the South African National Committee for Oceanographic Research. I am grateful to Dr Steve Blaber for his support and guidance as supervisor of this project and to the Natal Parks Board and KwaZulu Government for permission to work in areas under their control.

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