Short Paper

Rapid variation in kidney histology in spotted scat
Scatophagus argus on exposed to abrupt salinity changes

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Summary

In order to study the role of the kidney in fish osmoregulation, freshwater acclimatized juvenile spotted scat (Scatophagus argus L.) were subjected to four different salinities and observed for histomorphometric changes of the kidney at 1, 2, 10 and 15 days post transfer time points. The overall morphological changes displayed by fish kidney included significant decrease in the density of collecting tubules and glomeruli when subjected to higher salinity levels (10, 20 or 30 g/l) in comparison to proliferated, extensive, dense and muscular ones retained in the kidney of residents in freshwater and also of 5 g/l adapted animals. In conclusion, the observed histomorphological changes in the current study agree well with previously established physiological differences in the function of teleost kidney in freshwater and in the seawater.

Key words: Kidney, Spotted scat (Scatophagus argus), Osmoregulation, Glomerules, Collecting tubules

Introduction

A course of adaptation of teleosts to hyperosmotic enviroment involves coordinated physiological responses of several organs (Evans et al., 1999) with effective hydro-mineral regulations occurring mainly at the gill, the kidney, the intestine and the integument (Evans et al., 1999; Greenwell et al., 2003; Varsamos et al., 2005). Therefore, the kidney as an important osmoregulatory organ must play a major role in the survival of those called euryhaline teleosts such as the spotted scat (Scatophagus argus L.) as it contributes to routes maintaining the body fluid homeostasis during adaptation to different salinities (Cleveland and Trump, 1969; Karnaky, 1998). In saltwater (SW), the euryhaline fish kidney filters plasma at low rates to conserve water, and tubular secretion of electrolytes and fluid contribute significantly to urine formation, serving as the main secretory route for absorbed Mg2+, Ca2+ and SO42- (Cleveland and Trump, 1969; Karnaky, 1998). In freshwater (FW), the kidney filters at high rates and reabsorbs nearly all filtered solutes, thereby producing large volumes of dilute urine and in this way; the osmotic water loads of the FW habitat are excreted (Evans et al., 1999). Considering the above mentioned functional indentations, several histological changes must occur in the kidney during osmotic adaptation leading to alterations in excretive efficiency, glomerular filtration rate, and urine production in hyperosmotic or hypoosmotic environments (Beyenbach, 1995; Renfro, 1995). The present study used a comprehensive approach to study the effects of abrupt and chronic salinity shock on histomorphology of spotted scat kidney.
Materials and Methods

Study species
Approximately 200 Juvenile, first-generation hatchery-reared spotted scat (S. argus, L. 1766) obtained from Morvirid-Jonub ornamental fish trade Centre (Boushehr, Iran), where they were maintained in a closed freshwater system at 24-26°C temperature with a 12 h:12 h light/dark photoperiod and fed twice daily with 54% protein diet at 4% of the body weight. The fish were transferred to the wet laboratories at the Faculty of Sciences (Chamran University, Iran), where they were acclimated for 48 h to Ahvaz city tap water (referred to as fresh water) in 400-l tanks (n = 40 fish/tank), at the same temperature and photoperiod.

Experimental protocol
Five 250-l treatment aquaria situated in the same building as the acclimation tanks, were selected and filled with 5, 10, 20 or 30 g/l salt water or tap water as a blank treatment. The experimental salinities were achieved by diluting disinfected seawater (35 g/l) with fresh tap water (0.8 g/l) or by mixing dechlorinated tap water with natural marine salt, respectively. An acclimation tank was then randomly selected and the fish were anaesthetized by adding 40 ml/l clove oil to the tank water. Then 30 fish were transferred from the acclimation tank to a single, randomly selected treatment tank (one tank per one treatment aquaria). This process was repeated for each of the remaining acclimation and treatment tanks at 25 min intervals to ensure that all fish were exposed to anesthetic for the same length of time and to enable fish samples in individual treatment aquaria to be taken at the same time after transfer from freshwater. The experiment was conducted for 15 days, during which salinity in treatment tanks was maintained by adding fresh tap water as required. The fish were fed twice daily during the experiment with 54% protein at 4% of the body weight. Air was supplied to aquaria approximately 400 ml/min. Salinity, temperature (range 24.0-26.0°C), pH (range 8.2-8.6) and dissolved O2 (8.5-11.9 mg/l) were measured daily to the nearest 0.1°C, 0.1 pH unit and 0.1 mg/l, respectively using a water quality meter (Horiba U-10, Horiba Ltd, Japan).

Histological methods
During the experiment, the fish were sampled at the 1st, 2nd, 10th and 15th day post transfer (Before transfer, six fish were sampled and constituted the pre transfer control group). At each time point, n = 6 fish were netted and anaesthetized with Clove oil (ml/l). The trunk kidney was dissected and fixed in 10% formaldehyde in 0.1 M phosphate buffer (pH = 7.8). The tissue was then processed and embedded in paraffin using an automatic enclosed tissue processor (LEICA TP-1050). Afterwards the embedded tissues were cut into 5 µm sections, stained with hematoxyline and eosin and observed using a light microscope (Olympus, Model BX41, Tokyo, Japan) and images were photographed using a digital camera (Olympus, Model C-4040Zoom, Tokyo, Japan) which was mounted on the microscope. The number and diameter of glomeruli, the diameter of the collecting tubules and thickness of the muscular tissue of the collecting tubules were measured within areas where the glomeruli and collecting tubules were aggregated. A population of collecting tubules and glomeruli were calculated by counting the number of tubules in 10 random sites of fixed areas on each of the six sections. All measurements were made using a calibrated ocular micrometer.

Statistical analyses
All data were presented as means ± SE. Values were compared at P<0.5 significance level using one-way ANOVA followed by a tukey’s test.

Results
Kidney’s histology
Examination of the gross morphology of the kidney sections in spotted scat revealed structural differences between the experimental groups of fish. In general a mesonephric kidney type was identified. In some sections, a single afferent arteriol was seen to connect to several glomeruli. The
mesangium of the glomerulus in fish adapted to hypo-osmotic condition was more expanded (Fig. 1a), and the capillary lumens were wide within which red blood cells could be found. In contrast, mesangium in kidneys of fish adapted to hyper-osmotic environment appeared to be shrunken and collapsed and the capillary lumen was relatively small and inconspicuous (Fig. 1b, c, d, or e). The collecting tubules were morphologically distinct from the other tubules by the existence of constructed tall columnar epithelial cells enclosing the wide lumen, while proximal tubules were made up of cuboidal epithelial cells (Fig. 2b, c, d or e). In some sections, especially in those of hypo-osmatically adapted animals, foldings and invaginations were common in the collecting tubules, and the lumen appeared to be larger (Fig. 2a).

**Kidney morphometrics**

In the kidney of freshwater residents multiple glomeruli (three to six) with large lumen (72 µm in average) were found within each cluster of nephron (Fig. 1). In comparison, significantly decreased glomerular size and density were observed in saltwater adapted fish (Figs. 3a, b) and a significant relationship was revealed between time and glomerulus size during the saltwater adaptation course, but no significant relation could be demonstrated between time and glomerulus density in due order (Table 1). The collecting tubules also increased in number in fresh water residents and significant time dependant decrease in the populations of the tubules was recorded for animals subjected to salinity shock (Fig. 4a). Moreover, the lumen diameter of the collecting tubules varied from 55 in freshwater residents to 93 µm in saltwater adapted fish. Also, up to a twofold increase in muscular wall thickness was observed in the saltwater adapted fish (Figs. 4b, c). The kidney of animals adapted to 30 g/l salinity was similar to those subjected to 20 g/l saltwater in all respects, but some of the tubules were observed as not ending in a glomerulus unit.

**Discussion**

The teleostean kidneys are evolutionarily diverse and different forms have evolved independently. In the present study, the glomeruli were observed in the spotted scat kidney, convincing a general theme for vertebrate kidneys and most other teleosts. However, some other percomorphs like seahorse, pipefish, dragonet, sea poacher, scorpion fish, rock fish, puffer fish, porcupine fish, clingfish, and some

![Fig. 1: Glomerular morphology of spotted scat adapted to salinities of (a) 0, (b) 5, (c) 10, (d) 20 and (e) 30. Kidney sections were stained with H&E, (Scale bar = 40 µm)](image)

![Fig. 2: Collecting tubules morphology of spotted scat adapted to salinities of (a) 0, (b) 5, (c) 10, (d) 20 and (e) 30. Kidney sections were stained with HE lumen of collecting tubules. Both the diameter and the thickness of collecting tubules decrease in hyper-osmotic media, (Scale bar = 40 µm)](image)
Table 1: Time course of changes in glomerular and collecting tubules morphometrics in spotted scat subjected to abrupt hyper-osmotic exposure (salinity 0 to 30)

<table>
<thead>
<tr>
<th>Times (days)</th>
<th>Number of glomeruli (µm)</th>
<th>Glomeruli diameter (µm)</th>
<th>Number of collecting tubules (µm)</th>
<th>Collecting tubules thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.24 ± 0.13a</td>
<td>58.06 ± 2.9b</td>
<td>3.92 ± 0.36a</td>
<td>82.5 ± 3.66a</td>
</tr>
<tr>
<td>1</td>
<td>4.0 ± 0.14a</td>
<td>57.06 ± 3.8b</td>
<td>3.64 ± 0.19a</td>
<td>66.5 ± 3.38b</td>
</tr>
<tr>
<td>2</td>
<td>4.2 ± 0.112</td>
<td>49.07 ± 1.59b</td>
<td>3.42 ± 0.17a</td>
<td>67.5 ± 3.48b</td>
</tr>
<tr>
<td>10</td>
<td>3.95 ± 0.16b</td>
<td>49.95 ± 2.59b</td>
<td>3.5 ± 0.27a</td>
<td>66.4 ± 3.5b</td>
</tr>
<tr>
<td>15</td>
<td>4.13 ± 0.13b</td>
<td>48.03 ± 2.3b</td>
<td>3.0 ± 0.30a</td>
<td>64.0 ± 3.59b</td>
</tr>
</tbody>
</table>

Values are means ± SE (n=6). Glomerular and collecting tubules number did not significantly alter but glomerular diameter decreased during the exposure. Collecting tubules significantly decreased in diameter and thickness after exposure to hyper-osmotic. Different lower case letter indicates significant difference among different time intervals after one-way ANOVA (P<0.05)

Fig. 3: Glomerular morphometrics of spotted scat to different salinities showing (a) number of glomeruli and (b) mean glomerular diameter. Values are means ± SE (n=6). Number of glomeruli and glomerular diameter were significantly decreased in hyper-osmotic media. Different lower case letter indicates significant difference among different salinity groups after one-way ANOVA (P<0.05)

notherenoid antarctic fish may contain aglomerular kidney (Dobbs et al., 1974; Babiker and Rankin, 1979; Khalil, 1979). Indeed, environmental conditions as well as physiological adaptations may diminish the role of glomerular filtration in fish (Singer, 2001). In this case, the loss of body water in hyper-osmotic seawater and the contributions of the gills to extracellular fluid homeostasis may reduce the filtration demands on the kidney to such a degree to allow the dismissal of glomeruli in some fish (Singer, 2001). Moreover, unlike most teleostean kidneys consisting of dispersed glomeruli (Anderson and Loewen, 1975), the spotted scat kidney had both dispersed and clustered glomeruli. Wong and Woo (2006) also found four to six glomeruli in the silver sea bream kidney. The glomeruli of spotted scat adapted to different salinities...
Fig. 4: Collecting tubules morphometrices of spotted scat to different salinities showing (a) mean number, (b) mean diameter and (c) mean thickness of collecting tubules. Values are means ± SE (n=6). Diameter, thickness and number of collecting tubules were significantly decreased in hyper-osmotic media different lower case letter indicate significant difference among different salinity groups after one-way ANOVA (P<0.05).

were morphologically dissimilar. In strong hyperosmotic conditions mesangial tissues were shrunken, probably implying a greatly reduced single nephron glomerular filtration rate (Nebel et al., 2005). Its now well established that the reduction of the glomerular filtration rate to <10% of its rate in freshwater shifts the renal mechanisms of extracellular fluid homeostasis to the renal tubules in salt water (Brown et al., 1978; Furspan et al., 1984; Talbot et al., 1989) and glomeruli are not obliged to filter all the time, not even in freshwater fish. Also, glomerular shutdown in seawater fish does not mean renal shutdown or renal failure as it does in mammals (Beyenbach, 2004). In hypo-osmotic conditions (0 and 5 g/l) expanded mesenchymal tissues of dense glomeruli were observed. The same results were concluded when the euryhaline fish, Silver sea bream, was subjected to hypo-osmotic condition (Wong and Woo, 2006). Hyper-osmotic adaptation also resulted in decreased density of the collecting tubules of spotted kidney as well, which may be explained by tubule independent formation of urine in concentrated medium (Townsley and Scott, 1963). For convenience, Wong and Wu (1988) founded decreed areas of the collecting tubules in the euryhaline fish, Oreochro mossumbicus peters, kidney down to 26.5%. The collecting tubules in the study often had a single circular lumen devoid of any invaginations and folding in comparison to those adapted to freshwater as observed in the current study. It’s believed that, thicker and more luminous collecting tubules may facilitate a higher urine flow rate (Tsuneki et al., 1984). In general, the spotted scat responded rapidly to hyper-osmotic challenge. However, it seems that the osmoregulation process was not completed in the 15 day period.

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References


