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 Mg^{2+}, Ca^{2+} and SO_4^{2-} (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 O_2 (8.5-11.9 mg/l) were measured daily to the nearest 0.1%, 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 morphologicaly 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-osomatically 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 percomophs like seahorse, pipefish, dragonet, sea poacher, scorpion fish, rock fish, puffer fish, porcupine fish, clingfish, and some

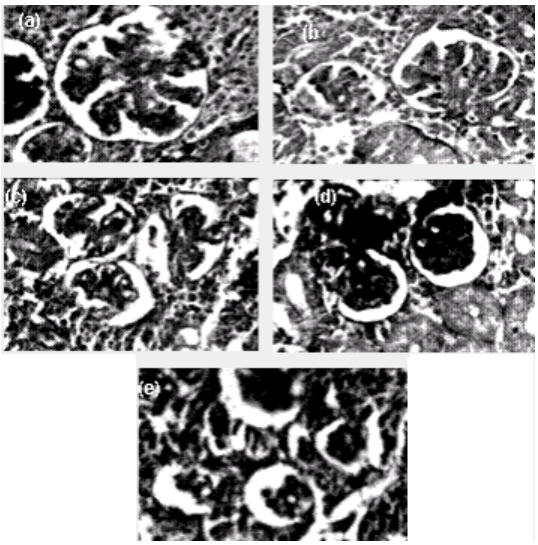


Fig. 1: Glomerular morphology of spotted scat adapted to salinities of (a) 0, (b) 5, (c) 10, (d) 20 and (e) 30. Kidney sections were stained with H&E, (Scale bar = 40 µm)

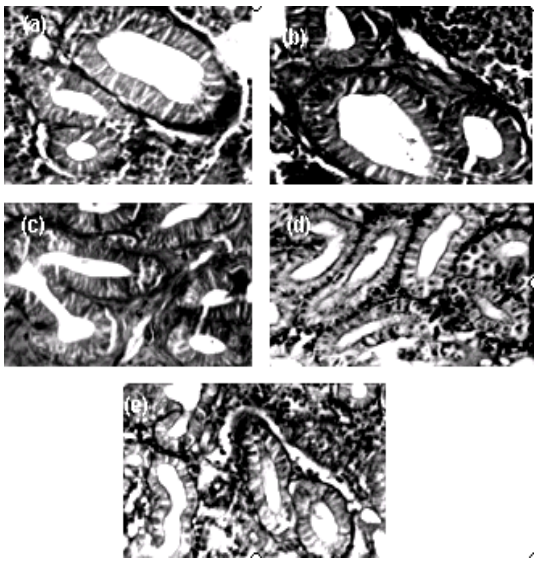


Fig. 2: Collecting tubules morphology of spotted scat adapted to salinities of (a) 0, (b) 5, (c) 10, (d) 20 and (e) 30. Kidney sections were stained with HE lumen of collecting tubules. Both the diameter and the thickness of collecting tubules decrease in hyper-osmotic media, (Scale bar = 40 µm)

Table 1: Time course of changes in glomerular and collecting tubules morphometrices in spotted scat subjected to abrupt hyper-osmotic exposure (salinity 0 to 30)

Times (days)	Number of glomerular	Glomeruli diameter (µm)	Number of collecting tubules	Collecting tubules diameter (µm)	Collecting tubules thickness (µm)
0	4.24 ± 0.13^{a}	58.06 ± 2.9^{a}	3.92 ± 0.36^{a}	82.5 ± 3.66^{a}	16.86 ± 0.53^{a}
1	4.0 ± 0.14^{a}	57.06 ± 3.8^{a}	3.64 ± 0.19^{a}	66.5 ± 3.38^{b}	12.10 ± 0.51^{b}
2	4.2 ± 0.11^{a}	49.07 ± 1.59^{b}	3.42 ± 0.17^{a}	67.5 ± 3.48^{b}	12.40 ± 0.42^{b}
10	3.95 ± 0.16^{a}	49.95 ± 2.59^{b}	3.5 ± 0.27^{a}	66.4 ± 3.5^{b}	11.87 ± 0.58^{b}
15	4.13 ± 0.13^{a}	48.03 ± 2.3^{b}	3.0 ± 0.30^{a}	64.0 ± 3.59^{b}	12.0 ± 0.50^{b}

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 ($P<0.05$)

notothenioid 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

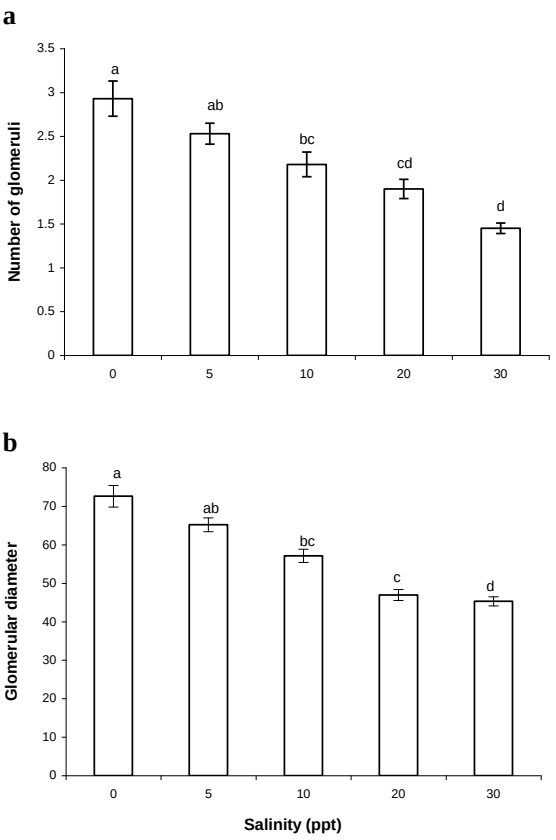
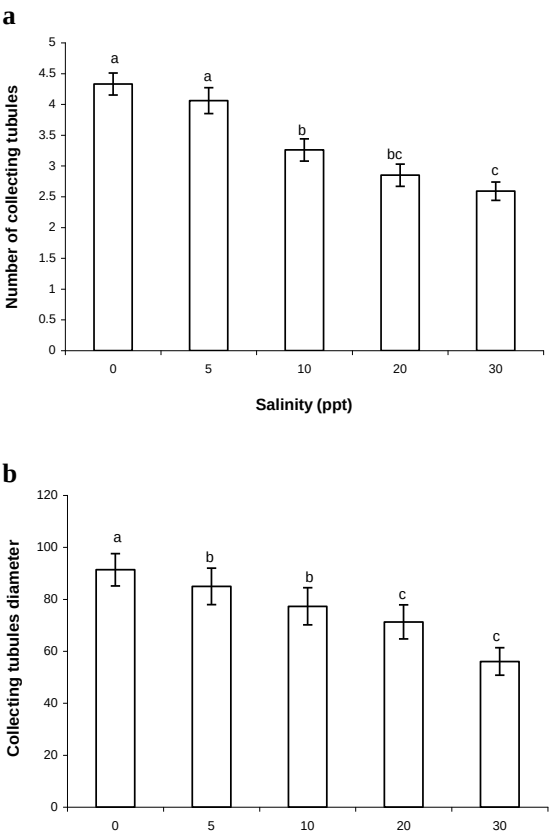


Fig. 3: Glomerular morphometrices of spotted scat to different salinities showing (a) number of glomeruli and (b) mean glomerular diameter. Values are means ± SE (n=6). Number of glomeruli and glomerular diameter were significantly decreased in hyper-osmotic media. Different lower case letter indicates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



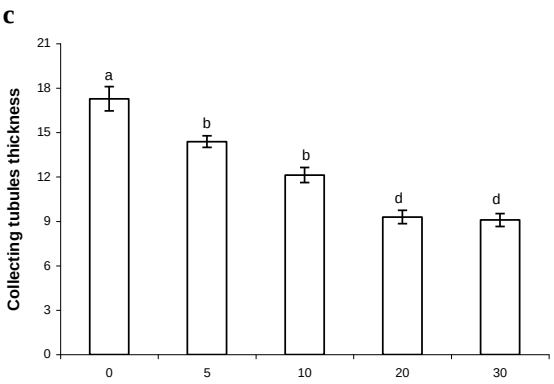


Fig. 4: Collecting tubules morphometrices of spotted scat to different salinities showing (a) mean number, (b) mean diameter and (c) mean thickness of collecting tubules. Values are means ± SE (n=6). Diameter, thickness and number of collecting tubules were significantly decreased in hyper-osmotic media different lower case letter indicate significant difference among different salinity groups after one-way ANOVA (P<0.05)

were morphologicaly dissimilar. In strong hyperosmotic conditions mesangical 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

Anderson, BG and Loewen, RD (1975). Renal morphology of freshwater trout. Am. J. Anat., 143: 93-114.

Babiker, MM and Rankin, JC (1979). Factors regulating the functioning of the *in-vitro* perfused aglomerular kidney of the anglerfish *Lophius piscatorius*. Comp. Biochem. Physiol., 62: 989-994.

Beyenbach, KW (1995). Secretory electrolyte transport in renal proximal tubules of fish. In: Wood, CM and Shuttlewoth, TJ (Eds.), *Fish physiology, ionoregulation: cellular and molecular approache*s. (3rd Edn.), New York, Academic Press. PP: 85-106.

Beyenbach, KW (2004). Kidneys sans glomeruli. Am. J. Physiol. Renal Physiol., 286: 811-827.

Brown, JA; Jackson, BA; Oliver, JA and Henderson, IW (1978). Single nephron filtration rates in the trout *Salmo gairdneri*. Validation of the use of ferrocyanide and the effects on environmental salinity. Eu. J. Physiol., 377: 101-108.

Cleveland, PH and Trump, BF (1969). The kidney. In: Hoar, WS and Randall, DJ (Eds.), *Fish physiology, excretion, ionic regulation and metabolism*. (1st Edn.), New York, Academic Press. PP: 91-239.

Dobbs, GH 3rd; Lin, Y and DeVries, AL (1974). Aglomerularism in antarcticfish. Science. 185: 793-794.

Evans, DH; Piermarini, PM and Potts, WTW (1999). Ionic transport in the fish gill epithelium. J. Exp. Zool., 283: 641-652.

Furspan, P; Prange, HD and Greenwald, L (1984). Energetics and osmoregulation in the catfish *Ictalurus nebulosus* and *Ictalurus punctatus*. Comp. Biochem. Physiol., 77:

773-778.

Greenwell, MG; Sherrill, J and Clayton, LA (2003). Osmoregulation in fish. Mechanisms and clinical implications. Vet. Clin. Exot. Anim., 6: 169-189.

Karnaky, JK (1998). Osmotic and ionic regulations. In: Evans, DH (Ed.), *The physiology of fishes*. (2nd Edn.), Boca Raton, New York, CRC Press. PP: 157-176.

Khalil, RM (1979). Histological studies on the kidneys of the red sea *Syngnathiformes* fishes. Zool. Soc. Egypt Bull., 29: 28-36.

Nebel, C; Romestand, B; Nègre-Sadargues, G; Grousset, E; Aujoulat, F; Bacal, J; Bonhomme, F and Charmantier, G (2005). Differential freshwater adaptation in juvenile sea-bass *Dicentrarchus labrax*: involvement of gills and urinary system. J. Exp. Biol., 208: 3859-3871.

Renfro, JL (1995). Solute transport by flounder renal cells in primary culture. In: Wood, CM and Shuttlewoth, TJ (Eds.), *Fish physiology, ionoregulation: cellular and molecular approaches*. (2nd Edn.), New York, Academic Press. PP: 147-173.

Singer, MA (2001). Of mice and men and elephants: metabolic rate sets glomerular filtration rate. Am. J. Kidney Dis., 37: 164-178.

Talbot, C; Eddy, FB; Potts, WTW and Primmett, DRN (1989). Renal functionin migrating adult atlantic salmon *Salmo salar* L. Comp. Biochem. Physiol., 92: 241-246.

Townsely, PM and Scott, MA (1963). Systolic muscular action of the kidney tubules of flounder. J. Fish. Res. Brd. Can., 20: 243-244.

Tsuneki, K; Kobayashi, H and Pang, PKT (1984). Electeron-microscopic study of innervation of smooth muscle cells surrounding collecting tubules of fish kidney. Cell tiss. Res., 238: 307-312.

Varsamos, S; Nebel, C and Charmantier, G (2005). Ontogeny of osmoregulation in post-embryonic fish: a review. Comp. Biochem. Physiol., 141: 401-429.

Wong, MKS and Woo, NYS (2006). Rapid change in renal morphometrics in silver sea bream (*Sparus sarba*) on exposure to different salinities, J. Fish. Biol., 69: 770-782.

Wong, HPP and Wu, SM (1988). Salinity effects on cytometrical parameters of the kidney in the euryhaline teleost *Oreochromis mossambicus* Peters. J. Fish. Biol., 33: 89-95.