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MORPHOLOGICAL STUDY ON KIDNEYS OF TILAPIA LARVAE (*OREOCHROMIS MOSSAMBICUS*) HATCHED IN FRESHWATER AND SEAWATER[1]

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Pung-Pung Hwang and Su-Mei Wu (1989) Morphological study on kidneys of tilapia larvae (*Oreochromis mossambicus*) hatched in freshwater and seawater. *Bull. Inst. Zool., Academia Sinica* **28**(2): 73-80. Morphology of nephrons and tubular cells in the kidneys of tilapia larvae (*Oreochromis mossambicus*) hatched in freshwater was compared with those hatched in seawater.

Both freshwater- and seawater-hatched larvae possess only one pair of nephrons which are composed of a renal corpuscle, a short neck segment, bisegmental proximal tubule segments with PAS-positive brush border, a straight distal tubule segment, a tall columnar epithelial collecting tubule segment and collecting duct. However in some cases of seawater-hatched larvae, vascular glomerulus is underdeveloped, and branching does not occur in collecting tubule.

The mean nuclear areas of renal tubules of the two groups of larvae are significantly different ($P=<0.01$ or 0.05). Comparing with the freshwater-hatched larvae, the seawater-hatched larvae decrease in the nuclear areas in all the segments of the nephron: 1st proximl, -37.5%; 2nd proximal, -36.6%; distal, -37.7%; collecting, -42.8%.

These morphological differences suggest a higher osmoregulation-requirements for freshwater-hatched larvae than seawater-hatched larvae.

Key words: Renal morphology, Teleost larvae, Osmoregulation.

Recent physiological studies have indicated that embryos and larvae of teleosts whose organ systems are still poorly developed, are able to osmoregulate *via* active transport (Guggino, 1980a, b). Gills and kidneys are the most important organs responsible for the osmoregulation in adult teleosts (Evans, 1979). Chloride cells in the larval skin perform the osmoregulation of larvae until the gills develop (Hwang and Hirano, 1985; Hwang, 1987, 1989). However, little is known of the function of the kidneys in the early development stages of teleosts.

In this study, histological and cytological observations were made on the kidneys of the tilapia larvae (*Oreochromis mossambicus*) hatched in freshwater and seawater. The morphological differences between two groups were suggested to be associated with their different osmoregulation-requirements.

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MATERIALS AND METHODS

Newly hatched larvae of tilapia (*Oreochromis mossambicus*) were obtained with the same method described in the previous study (Hwang and Sun, 1989). Mature adults were reared in fresh water or 30-34‰ salinity sea water for over one month. Fertilized eggs were collected from the mouth of female which has just started mouth breeding. The eggs were incubated in running and aerated freshwater or seawater. Larvae were sampled within 24 h after hatching.

After being anesthetized with MS222, the larvae were fixed in Bouin's fixative for 12-24 h. Paraffin sections of 3-5 μm thickness were prepared and stained in (1) Periodic-Acid-Schiff counterstained with Mayer's hematoxylin and orange G, (2) Mayer's hematoxylin and eosin.

Serial cross or longitudinal sections of the whole larva were prepared and examined with a Nikon Optiphot optic microscope. Cytometric measurements were carried out with a micrometer insered into the occular lens. The nuclear diameters of renal tubules referred to their maximal dimensions in the plane of section. Nuclear area was calculated from its diameter through a formula for the area of a circle: $S=1/4\times D^2\times 3.14159$ (S, transverse area; D, diameter). 100 nuclei of each larva (20-30 nuclei each kind of segment) were measured. Studnt's T test was applied for statistical analysis.

RESULTS

Right after hatching, both freshwater- and seawater-hatched tilapia larvae still are poorly developed in organ systems, possessing a large oval-shaped yolk sac, brain with large cerebral ventricle, a gill rudiment, a straight gut and a kidney which composes of only a pair of nephrons.

Freshwater-Hatched Larvae

The nephron of a larva consists of renal corpuscle, a neck, two proximal tubules, a distal tubule, a collecting tubule and collecting duct (Figs. 1-6). However, characteristics of various segments in larval nephron are not as evident as those in adult (Hwang and Wu, 1987).

Renal corpuscle, neck, and two segments of proximal tubule occur near the head region (Fig. 1). Main part of renal corpuscle is a ramifying capillary, i.e., vascular glomerulus, which is surrounded with Bowman's capsule (Fig. 1). Neck segment is very short. Cells of this segment pack tightly and possess strong basophilic cystoplasm. The cell size is larger in proximal segments than that in neck segment. In some sections, PAS-positive brush border occured in both first and second proximal segments, however first proximal segment with basally located nuclei and supranulear granules could be distinguished from second proximal segment (Fig. 2).

Proximal segments appear to wind

Figs. 1-6. Micrographs of nephrons in freshwater-hatched tilapia (*Oreochromis mossambicus*). All the scales=20 μm.

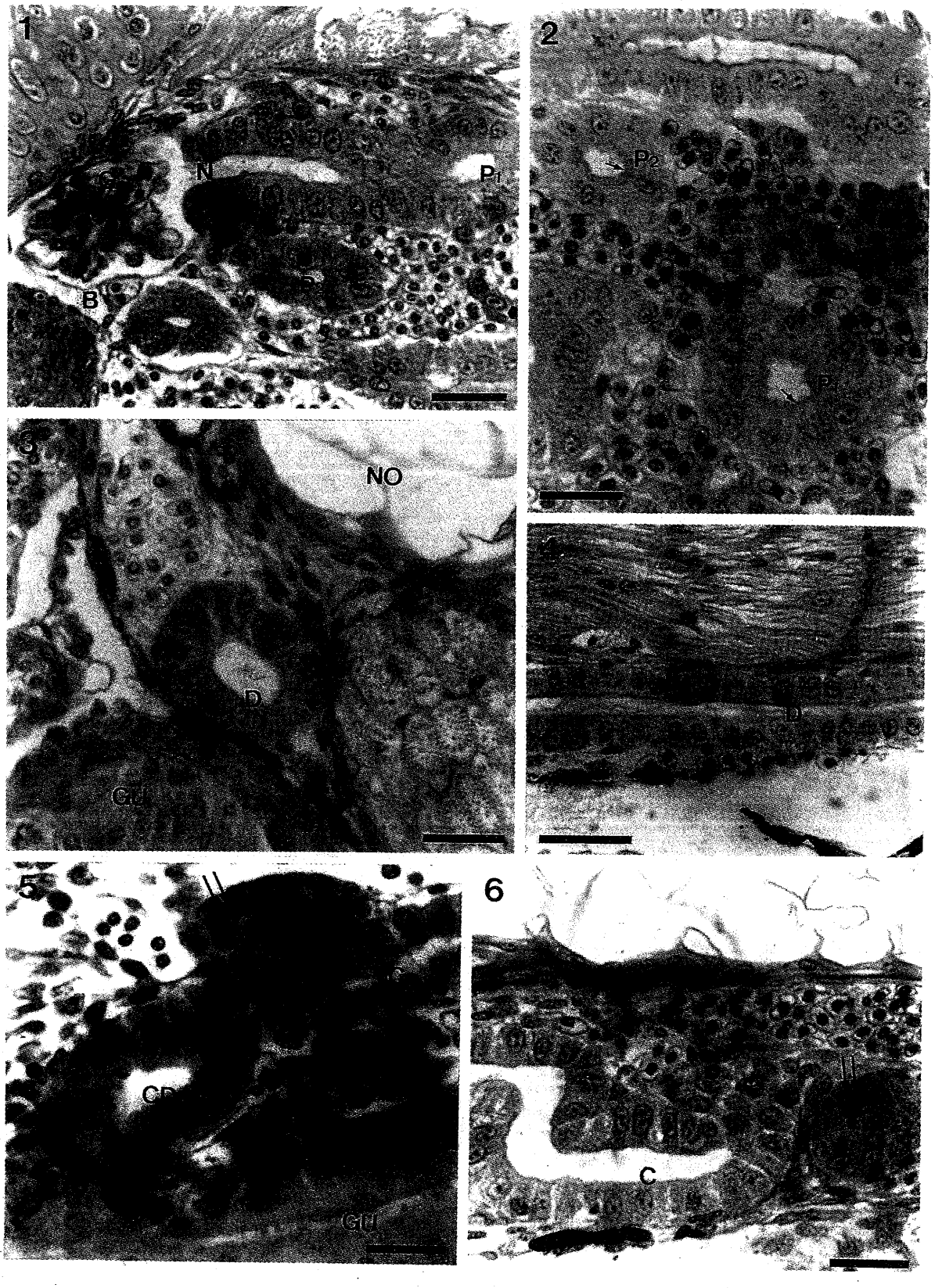
Fig. 1. Cross section of head region to show glomerulus (G), first proximal (P1) and second proximal segments (P2). B, Bowman's capsule; N, neck segment. HE.

Fig. 2. PAS-positive brush border (arrows) to be noted in P1 and P2.

Fig. 3. Cross section of distal segment (D). GU, gut; NO, notochord. HE.

Fig. 4. Longitudinal section of body to show straight distal segment (D). PAS.

Figs. 5 and 6. Longitudinal sections to show collecting tubule (C) and collecting duct (CD). Note the branching areas (double arrows) near collecting tubule. HE in Fig. 5, PAS in Fig. 6.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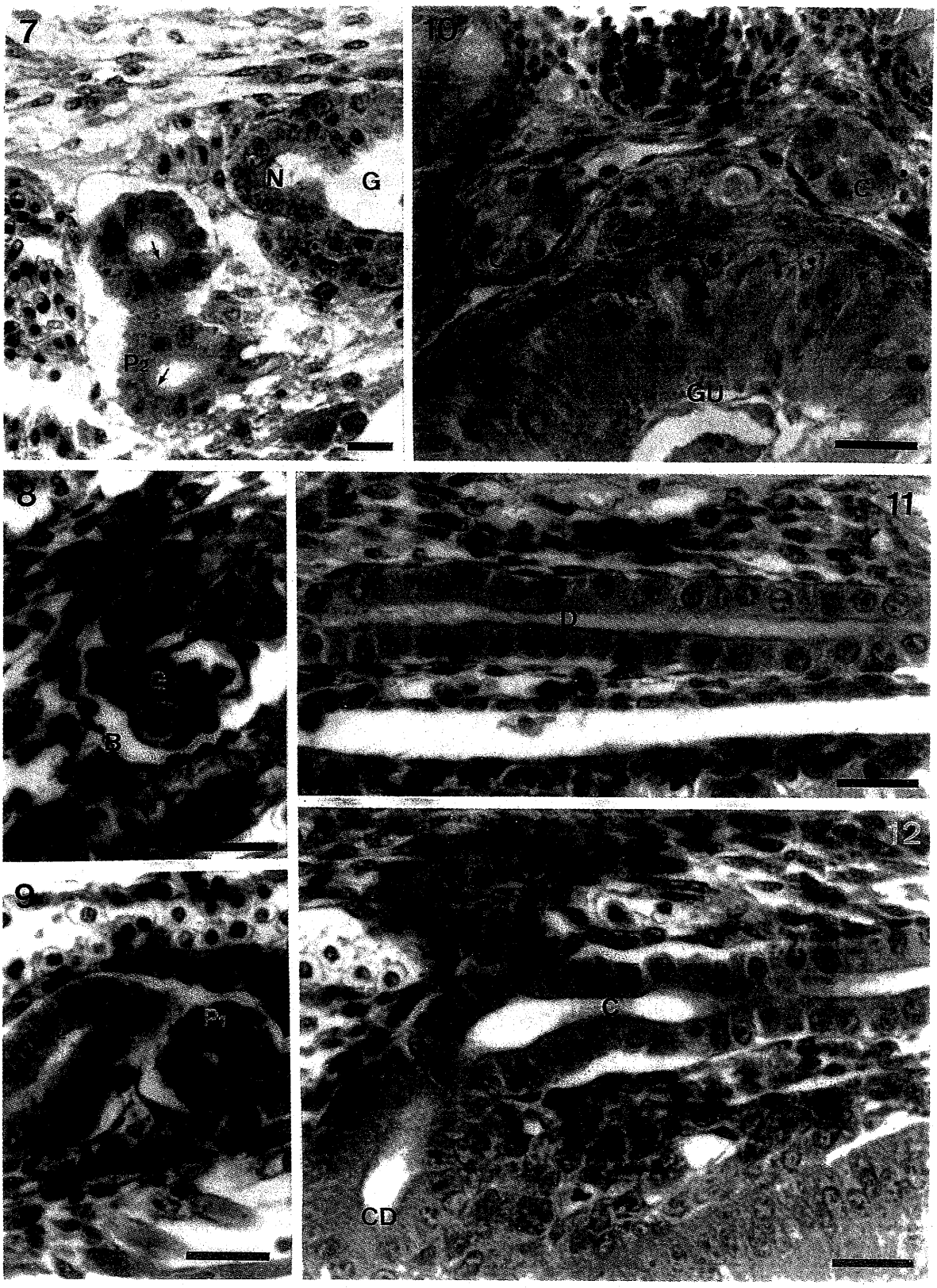
7
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their ways (Figs. 1 and 2), while distal segment extends straightly between notochrd and gut (Figs. 3 and 4). The morphology of tubular cells between posterior part of second proximal and distal is similar (Figs. 1-4), but distal segment could be confirmed by the location since it is anterior to collecting tubule.

As in distal segment, collecting tubule does not possess PAS-positive brush border (Figs. 3 and 6), however collecting tubule is easy to distinguished from distal segment since the former is lined by taller columnar cells (Figs. 5 and 6). Moreover, bending and branching are generally found in this segment (Figs. 5 and 6).

Similar to collecting tubule, collecting duct is lined by columnar cells whose nuclei locate at the base of cell, however collecting duct, being the last segment of nephron, shows much taller in cell height (Figs. 4-6).

Seawater-Hatched Larvae

The basic morphology of the nephrons of seawater-hatched larvae (Figs. 7-12) is similar to that of freshwater-hatched larvae (Figs. 1-6). The nephrons of seawater-hatched larvae also contain renal corpuscle, a neck, two proximal tubules, a distal tubule, a collecting tubule and collecting duct (Figs. 7-12), however they appear to be less developed than those of freshwater-hatched larvae.

In some cases, the vacuolar glomeruolus is not yet developed, i.e., the renal corpuscle shows only a hollow structure (Fig. 7). Proximal segments generally contain PAS-positive brush border (Fig. 7), but some of them show much basophilic cytoplasm (Fig. 9).

Morphological distinction among distal, collecting tubule and collecting duct in the nephron of seawater-hatched larva is similar to that of freshwater-hatched larva. Distal segment lined by columnar or cuboidal epithelium, extends its way straightly (Figs. 10 and 11), while collecting tubule and collecting duct are composed of much taller columnar cells (Fig. 12). Bending and branching in the collecting tubule of freshwater-hatched larva seldom could be found in the same segment of seawater-heatched larva (Fig. 12).

Cytometrical Comparision

In both groups, nuclear areas are similar in various segments. The nuclear areas of renal tubular cells in freshwater-hatched larvae are about 21-23 μm^2, while in seawater-hatched larvae are about 13-14 μm^2 (Table 1). Obviously, significant differences ($P = <0.01$ or 0.05) exist between the mean nuclear area of renal tubules of the two groups of larvae (Table 1). Comparing with the freshwate-hatched larvae, the seawater-hatched larvae reveal evident decrease in the nuclear areas of all the four main segments of the nephron: 1st proximal, −37.5%; 2nd proximal, −36.6%; distal, −37.7%; collecting, −42.8%.

Figs. 7-12. Micrographs of nephrons in seawater-hatched tilapia (*Oreochromis mossambicus*). All the scales =20 μm. HE in all figures.

Fig. 7. Proximal segments (P1 and P2) possessing brush border (arrows) and renal corpuscle with underdeveloped glomerulus (G). N, neck segment.

Fig. 8. Developed glomerulus (G) and Bowman's capsule (B).

Fig. 9. Proximal segments (P1 and P2).

Fig. 10. Cross section of body, to show one pair of nephrons. C, collecting tubule; GU, gut.

Figs. 11 and 12. Longitudinal sections of body to show straight distal segment (D), collecting tubule (C) and collecting duct (CD).

Table 1
Comparision of nuclear areas (μm^2) of the renal tubules in larvae of *Oreochromis mossambicus* hatched in freshwater and seawater. Mean±SD is given

	1st Proximal	2nd Proximal	Distal	Collecting
Freshwater	22.88±1.18	21.55±2.46	20.92±2.86	21.89±4.05
N	4	5	3	4
Seawater	14.30±2.20[a]	13.66±3.09[a]	13.03±1.16[b]	12.51±0.28[b]
N	4	6	4	3

a. Significant difference (P=<0.01)
b. Significant difference (P=<0.05)

DISCUSSION

Right after hatching, tilapia larvae (*Oreochromis mossambicus*) have developed a pair of nephrons which differentiated into various segments similar to those of adult (Hwang and Wu, 1987). Similar observations have been done in several species. For example, in pink salmon (*Oncorhynchus gorbuscha*), development of mesonephros was started in embryo stage (Ford and Newstead, 1958), and well-developed mesonephrons which consisted of glomerulus, three major segments and collecting duct were observed in fry stage (Newstead and Ford, 1960), while in striped bass (*Roccus saxatilis*) newly hatched larvae possessed an aglomerular pronephric kidney (Bryant, 1970).

It is worthy to note from the present results that freshwater-hatched tilapia larvae possess a more developed nephron than those of seawater-hatched larvae, i.e., some partialy developed glomeruli and non-branched collecting tubules occur in seawater larvae. Bryant (1970) reported that during the early larval stages of striped bass (*Roccus saxatilis*), development of mesonephric kidney is characterized by a decrease in salinity tolerance levels. As Tay and Garside (1978) suggested, it appears that differential growth of glomeruli and tubules is probably adaptive, being governed by physiological demands during embryogenesis and early larval growth.

It is evident that upon salinity adaptation, teleost adults including tilapia (*Oreochromis mossambicus*), exhibit their adaptive responses, including cytometric changes in tubular cells which are indicative of profound changes in kidney functions (Ogawa, 1968; Hickman and Trump, 1969; Wendelaar Bonga, 1973; Stevens and Bick, 1975; Olivereau and Olivereau, 1977; Tay and Garside, 1978; De Ruiter, 1980; Colville *et al.*, 1983; Hwang and Wu, 1988). Using the single nephron GFR method, Brown *et al.* (1978; 1980) indicated that about 10 folds of difference in GFR between freshwater and seawater rainbow trout (*Salmo gairdneri*) were ascribed to massive differences in the numbers of functional tubules. Thus, the larger nuclear areas of tubule cells of freshwater fish were suggested to be concerned with the much more functional tubules in the freshwater fish (Hwang and Wu, 1988). These seem also to hold in the newly hatched larvae of tilapia, since the nuclear areas in seawater-hatched larvae are only 55-65% of freshwater-hatched larvae (Table 1).

Much work remains to be done to elucidate the whole osmoregulation mechanism in the early development stages of teleosts. However, the present work and the previous studies (Hwang

and Hirano, 1985; Hwang, 1987; Hwang, 1989) have indicated that teleost larvae may perform osmoregulation function *via* one pair of nephrons and skin chloride cells.

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淡、海水吳郭魚 (*Oreochromis mossambicus*) 孵化仔魚腎臟形態學之研究

黃 鵬 鵬　　吳 淑 美

本報告比較淡水，海水吳郭魚 (*Oreochromis mossambicus*) 孵化仔魚腎臟的型態。

淡水與海水的孵化仔魚均只具有一對腎元，這個腎元是由一個腎小體，一段短的頸節，二段具有PAS陽性刷狀緣的近位細尿管、一段遠位細尿管、一段高栓狀上皮之集合細尿及集合管所組成。然而，一部份海水仔魚的絲球體尚未分化，而且在集合細尿管也沒有分枝的現象。

淡水及海水孵化仔魚的腎小管的細胞核面積有顯着的不同，海水孵化仔魚的各段細胞核面積都比淡水的孵化仔魚小。第一段近端細尿管：減少 37.5%，第二段近端細尿管：減少 36.6%，遠端細尿管：減少 37.7%，集合細尿管：減少 42.8%。

這些型態上的差異，被推測與淡、海水孵化仔魚調節滲透壓上不同之需求有關連。