COMPARATIVE HISTOLOGY OF THE KIDNEY OF Oligosarcus jenynsi AND Rhamdia sapo FROM LOS PADRES LAKE (BUENOS AIRES PROVINCE, ARGENTINA)

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RESUMEN

El "dientudo" *Oligosarcus jenynsi* y el "bagre sapo" *Rhamdia sapo* son dos especies de peces dulceacuícolas que habitan en la Laguna de Los Padres. Se han estudiado las estructuras histológicas del tejido excretor del riñón con el fin de establecer tanto los componentes como las características de sus nefronas. Como en la mayoría de otros peces teleósteos de agua dulce, la nefrona se compone de corpúsculo renal, segmento del cuello, segmento proximal y segmento distal. Los corpúsculos renales son grandes y abundantes y presentan glomérulos con una red capilar bien desarrollada. El segmento proximal se divide en dos porciones: el segmento proximal primario y el segmento proximal secundario. El segmento distal está bastante desarrollado. El riñón de ambas especies está adaptado a la necesidad de eliminar el exceso de agua para evitar una hidratación excesiva.

Palabras claves: teleósteos- dientudo- bagre- riñón- histología.

ABSTRACT

The histological structures were studied in the excretory tissue of the kidneys of two freshwater fish *Oligosarcus jenynsi* and *Rhamdia sapo* from Los Padres Lake (Buenos Aires Province, Argentina) in order to assess the components as well as the characteristics of their nephron. The nephron is made up of renal corpuscles, neck segment and the proximal and distal segments, as in most freshwater teleosts. Both species exhibit large and abundant renal corpuscles, containing glomeruli with a well developed capillar net. The proximal segment is divided in two fragments: the primary and secondary segments. A well - developed distal tubule was observed. The kidney of both species is adapted to get rid of the osmotic water loads, in order to avoid an excesive hydration.

Key words: teleost- characid- catfish- kidney- histology.

INTRODUCTION

The kidney is considered the main osmoregulatory organ of teleosts fish, carrying out the homeostasis maintenance, together with extrarenal mechanisms, which operate in gills and intestine. In euryhaline fish, the kidney has to keep up this balance in both fresh and marine waters. Some of them (e.g. the "striped bass" and the "tilapia") have been studied from the histophysiological point of view (Groman, 1982; Kamunde and Kisia, 1994; Balm *et al*, 1995).

Freshwater fish have a greater osmotic pressure than the surrounding waters, so that they develop renal and branchial mechanisms to filter water and to absorb salts, respectively (Kamunde and Kisia, 1994; Díaz et al, 1996). *Oligosarcus jenynsi* (Characidae) and the "catfish" *Rhamdia sapo* (Pimelodidae) frequently inhabit the shallow and polymíctic lakes from the so-called depressed Pampa (Argentina), playing different roles in trophic webs. *O. jenynsi* uses to live near submerged macrophytes, where it feeds on, while *R. sapo* is associated to the bottom (Ringuelet *et al*, 1967; Ringuelet, 1975).

Los Padres lake is one of these water bodies, situated at 37° 56' 30" S and 57° 44' 30" W (in the Buenos Aires province, Argentina), with an area of 2.16 km^2 and 1.24 m of mean depth (Pozzobon and Tell, 1995; Campana *et al*, 2001), and where these two species are frequently found (Martínez *et al*, 1995).

This pond represents a very attractive place for tourism, sports, and fishing (Escalante *et al*, 1998). Its watershed includes one affluent -named Los Padres creek- which flows through an important agricultural-livestock area, wherein great amounts of pesticides are applied, and one effluent -named La Tapera creek- running toward the sea (González Sagrario *et al*, 1998, 2002; Miglioranza *et al*, 1999, 2002).

Within the frame of a wider investigation on the trophic status of Los Padres lake, the aim of the present work is to study the histological structures of the excretory tissue of *Oligosarcus jenynsi* and Rhamdia sapo kidney, in order to assess the components as well as the characteristics of their nephron.

MATERIALS AND METHODS

Oligosarcus jenynsi (Lt = 121-175 mm) and *Rhamdia sapo* (Lt = 335- 430 mm) of both sexes were studied. The specimens were collected alive from Los Padres lake, and immediately carried to the laboratory and then killed by a sudden blow on the head.

For histological study, the kidneys were removed and fixed with 10% formalin and formol-buffer as follows: 1000 ml formalin 10%, 4 g sodium acid phosphate, 6.5 g anhydrous disodium phosphate (pH 7.4).

The material was embedded in paraffin, and cut in 4-5µm thick sections and stained with hematoxylin and eosin (H/E), Masson's trichrome stain (Humason, 1962), Periodic acid- Schiff (PAS) for glycoproteins (GPs) with oxidizable vicinal diols and/ or glycogen, and Alcian Blue (AB) pH 2.5 for GPs with carboxyl groups (sialic acid or uronic acid) and/or with sulphate esters (Martoja and Martoja-Pierson, 1970).

In sections stained with H/E, the diameter of corpuscles and glomeruli was estimated. The mean of two measurements, taken at approximately 90° angles with respect to the corpuscular center, was used.

T-test was used to compare diameter media between corpuscles and glomeruli of characid and catfish respectively. P-value was compared to a signification level of 5%. BMDP program sofware was used (Sokal and Rohlf,1979).

Photographs were taken using an Olympus CH 30 microscope, with a 100 ASA film.

RESULTS

The kidney of *O. jenynsi* and *R. sapo* is covered by a thin capsule of connective tissue with collagenous fibers. The renal parenchyma is not organized in cortex and medulla. Nephrons, collecting tubules, opisthonephric ducts, and the hematopoietic tissue are components of the kidney (Fig. 1). In both species, the nephron is composed of the renal corpuscle, the neck segment, the proximal segment, and the distal convoluted segment.

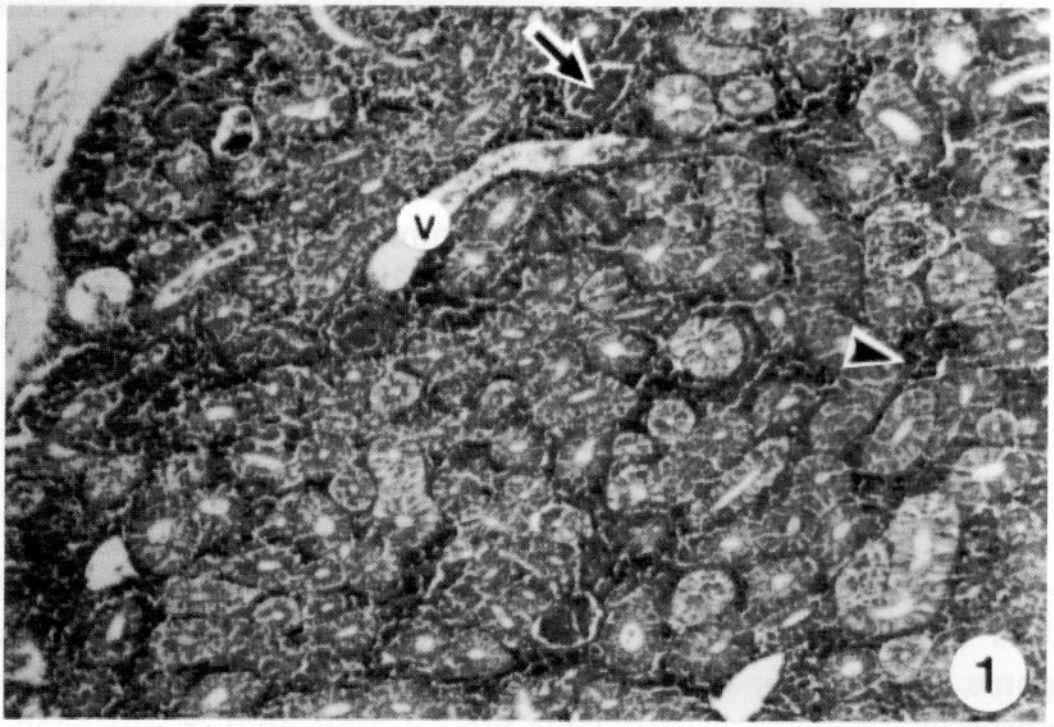

Fig. 1: Kidney of Rhamdia sapo. Renal corpuscle (arrow). Blood Vessels (v). Hematopoietic tissue (arrowhead). H/E (Magnification: X 33).

Renal corpuscles: they show a basic construction similar to other vertebrates, with vascular capillary glomeruli enclosed in the Bowman capsule, and scattered in the whole parenchyma (Fig. 2).

According to the glomerular classification of Marshall and Smith (1930, in De Ruiter 1980), *Oligosarcus jenynsi* and *Rhamdia sapo* belong to group I, as they exhibit large and abundant renal corpuscles.

Significant difference was found applying t-test between corpuscles and glomeruli medias in each specie. However there was no evident

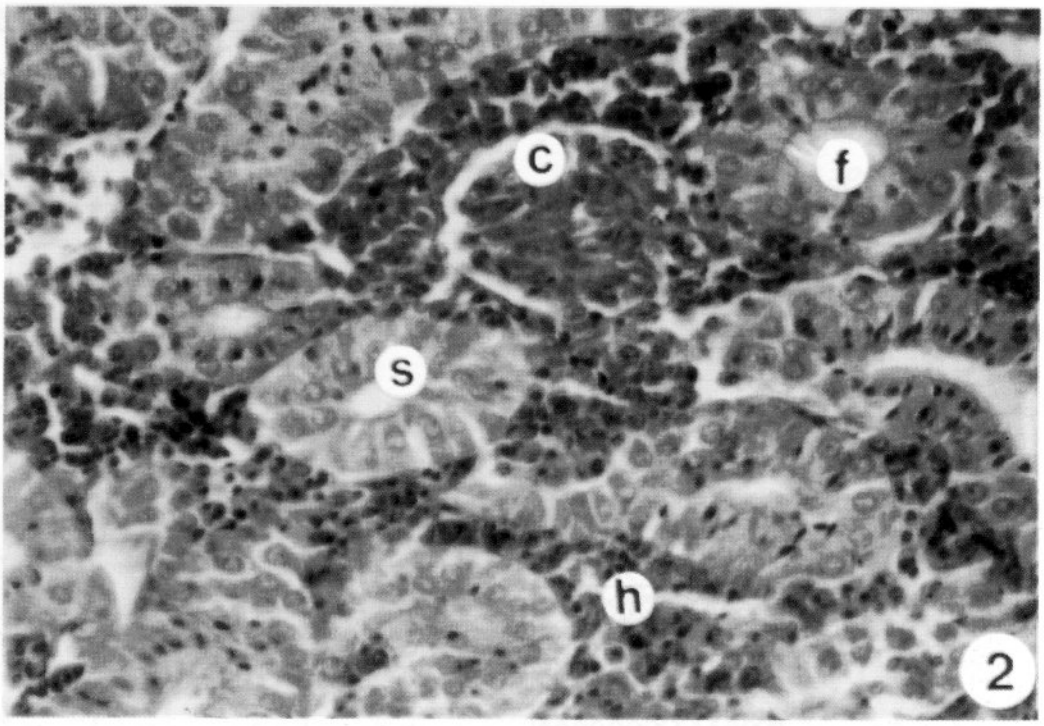

Fig. 2: Kidney of Rhamdia sapo. Renal corpuscle (c). Hematopoietic tissue (h). First segment of the proximal tubule (f). Second segment of the proximal tubule (s). H/E (Magnification: X 132).

differences between corpuscles diameter media between *Oligosarcus jenynsi* and *Rhamdia sapo*, neither between glomeruli of both species (Table I).

Species	Corpuscle (µm)	Glomerulus (µm)
O. jenynsi (90)	**92.96 ± 16.40 [66.15; 138.6]**	**77.14 ± 16.98 [44.1; 126]**
R. sapo (90)	**97.44 ± 14.80 [66.15; 126]**	**78.61 ± 15.23 [47.25; 122.85]**

Values are given as mean ± SD. The number of samples is given in parentheses.
Minimun and maximun diameter values appear between brackets.

Table I: Diameter of corpuscles and glomeruli

Moreover, corpuscles and glomeruli diameter of these two species does not statistically differ from that of other freshwater teleosts.

Neck segment: it consists of a very short tubule with low cuboidal cells and basal nuclei, and located at the glomerulus-tubule junction.

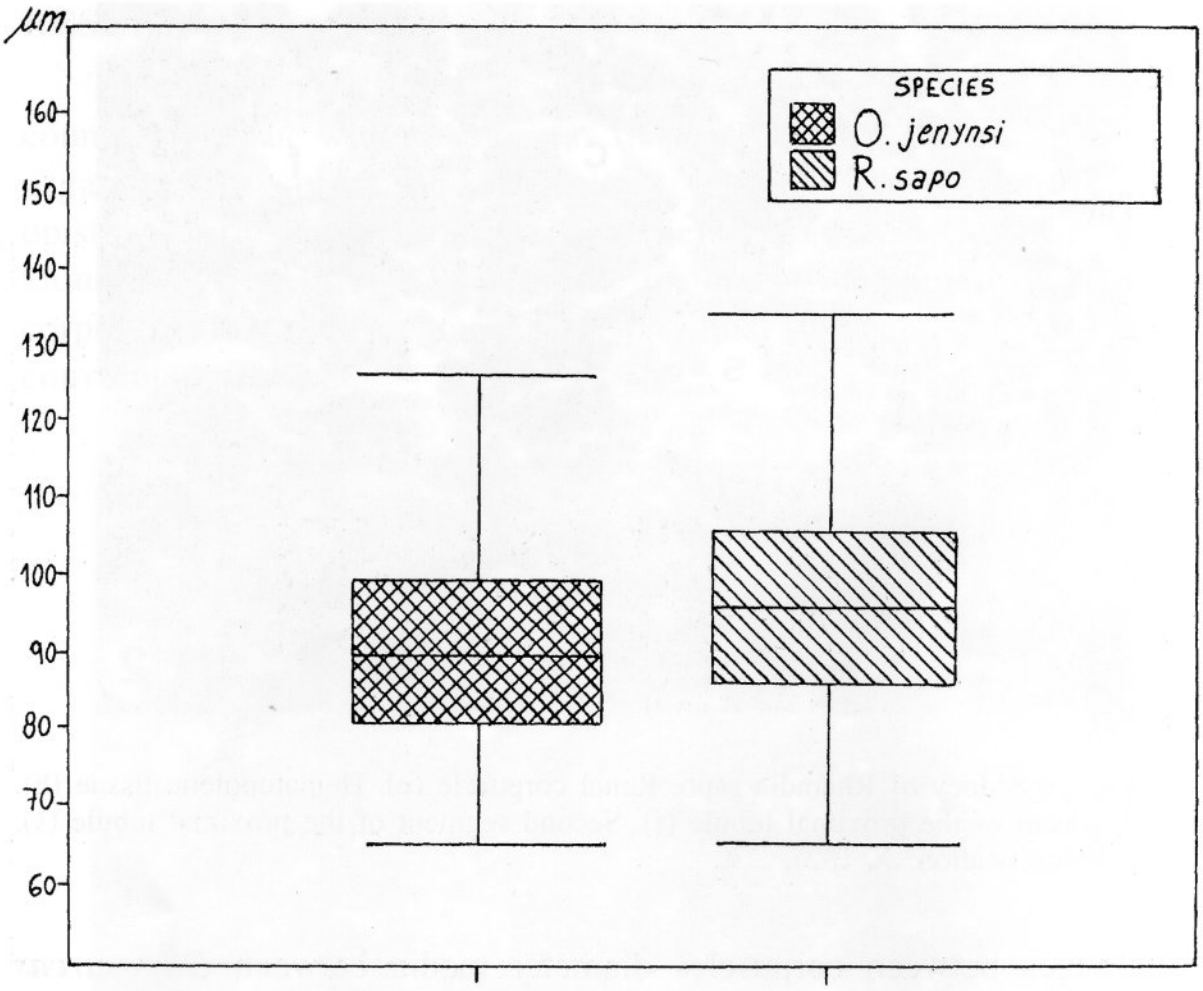

Fig. 3: Boxplot of the variable corpuscles for O. Jenynsi and R. sapo. Crossbars inside the rectangle represent the median. The limits of the rectangle are the quartiles. The whiskers show the range between minimum and maximun observations.

The transition from the neck segment to the proximal segment is abrupt and marked by an increase in cellular height and appearance of microvilli in the apical region (Fig. 3).

Proximal segment: it is divided in two fragments, the primary and secondary segments. The initial part of the proximal segment is composed of columnar cells with basal round nuclei. Its lumen is relatively wide, and has a brush border, showing a positive reaction for PAS.

The typical cell of the second part of the proximal segment is a high columnar acidophilic cell with median to apically located oval nucleus. It is a small tube where a reduction of the brush border length and thickness is observed, showing a weak positive reaction for PAS (Fig. 2).

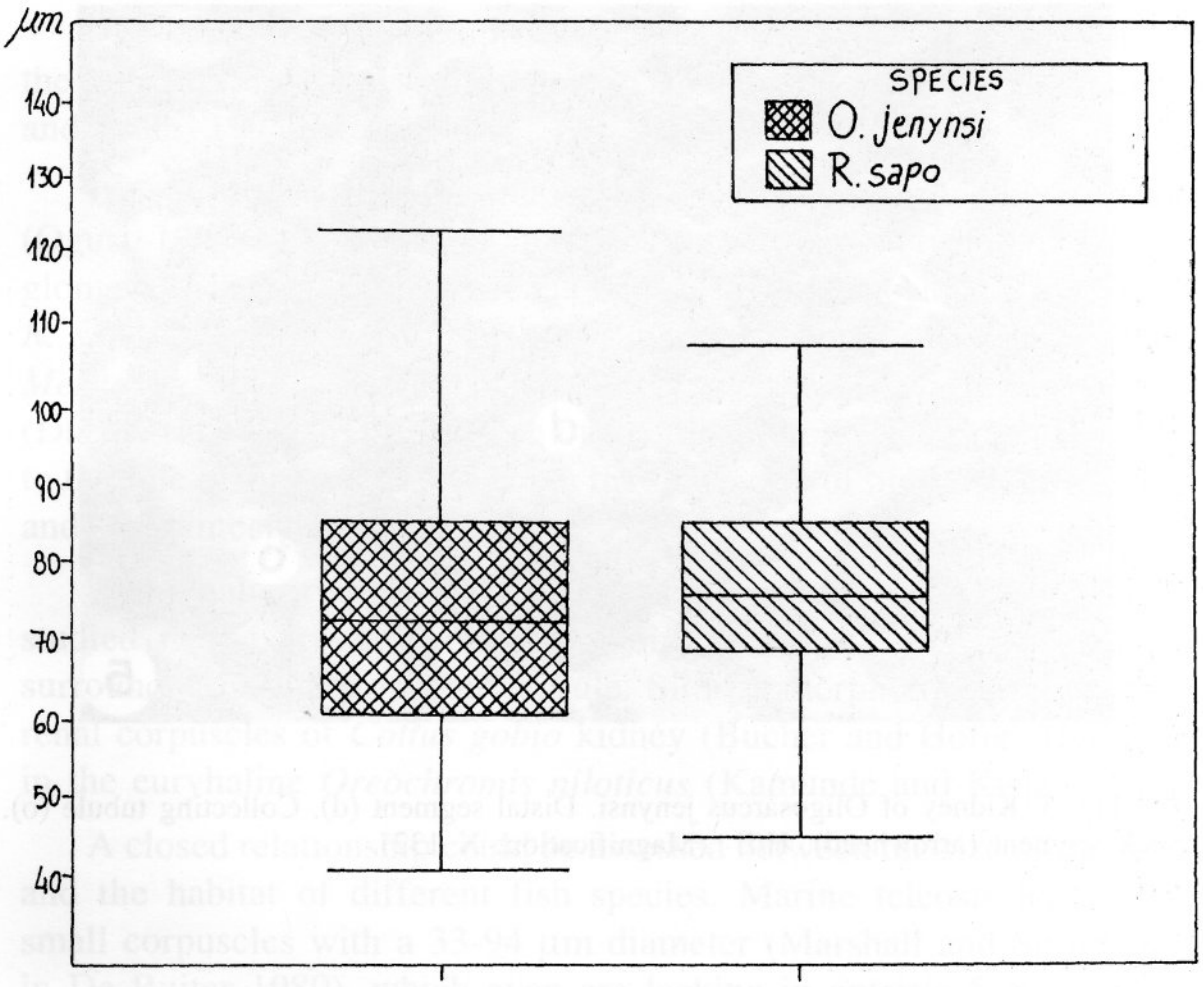

Fig. 4: Boxplot of the variable glomeruli for O. jenynsi and R. sapo. Crossbars inside the rectangle represent the median. The limits of the rectangle are the quartiles. The whiskers show the range between minimun and maximun observations.

Distal segment: it presents a narrow lumen and low columnar cells with a round nucleus located in the basal half of the cell. Cellular boundaries are well defined and without brush border (Fig. 3).

Collecting tubule: the epithelium may vary from simple cylindric to pseudostratified form, and is surrounded by a layer of fibrillar connective tissue (Fig. 3). Boundaries among cells are well defined, and the apical membrane weakly reacts for PAS and AB (pH 2.5). In *R. sapo*, the apical face of the cell is furnished with cilia.

Opisthonephric duct: it has a wider lumen than the collecting tubule, pseudostratified epithelium with basal nuclei, and the amount of connective tissue that surrounds the duct is increased.

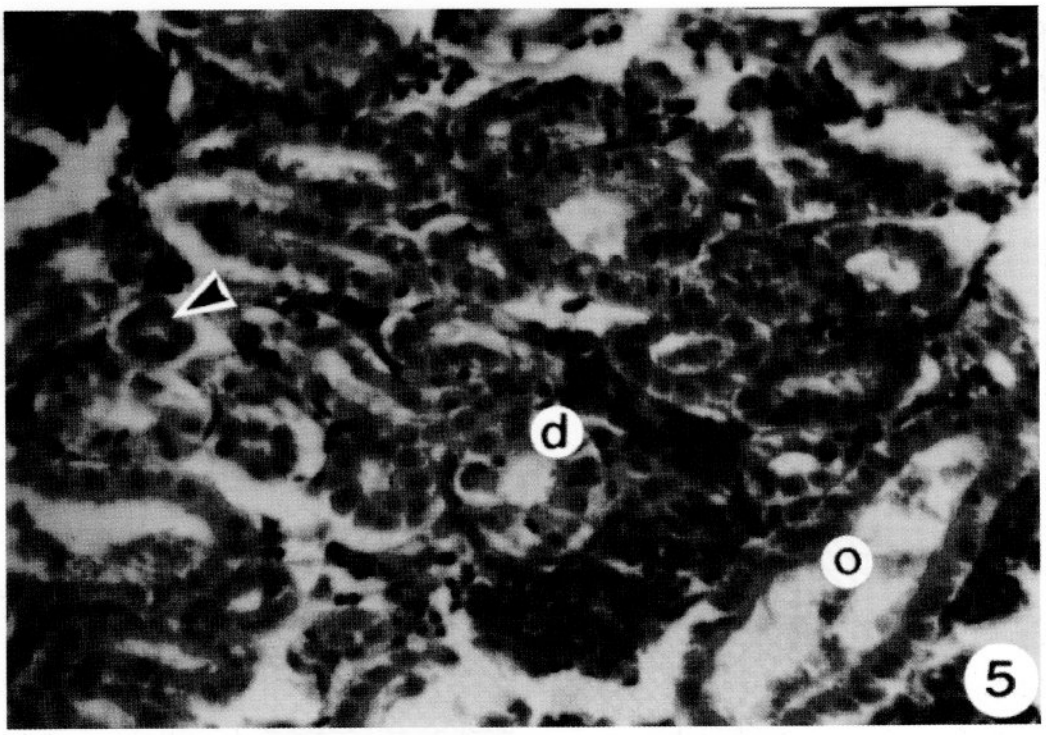

Fig. 5: Kidney of Oligosarcus jenynsi. Distal segment (d). Collecting tubule (o). Neck segment (arrowhead). H/E (Magnification: X 132).

Interstitial tissue composed of well developed hematopoietic tissue is observed in the renal parenchyma (Fig. 1). It includes hemoblast, lymphocytes, and macrophages. Some of the latter are grouped in melanomacrophage centers, characterized by the presence of several pigments.

Both, in *O. jenynsi* and *R. sapo*, lymphocytes are regularly arranged between epithelial cells near the lumen of renal tubules. These cells have small and dark nuclei which are spherical or oblong.

DISCUSSION

The structural organization of *Oligosarcus jenynsi* and *Rhamdia sapo* kidneys is basically similar to that of other teleostean fishes. A cortex and a renal medulla cannot be histologically distinguished, and the nephrons, the collecting tubules, and the interstitial tissue are the main components of the trunk kidney tissue (Oguri, 1987; Díaz *et al*, 1996).

The nephron is made up of the renal corpuscle, neck segment, and the proximal and distal segments, as in most freshwater teleosts (Kamunde and Kisia, 1994).

In teleostean fishes the interstitial tissue is generally hematopoietic (Oguri, 1987), and forms an abundant parenchyma between tubules and glomeruli. This is the case for the two freshwater species *O. jenynsi* and *R. sapo*, and was also observed in marine species, such as the "hake" *Merluccius hubbsi*, and the "white croaker" *Micropogonias furnieri* (Díaz *et al*, 1996). The function of the interstitial tissue might be related to the role that plays the kidney in the formation of blood elements (Díaz and Goldemberg, 1998).

The renal corpuscle histology is similar in both freshwater species studied in the present work, containing glomeruli well irrigated, and surrounded by the Bowman capsule. Similar morphology was found in renal corpuscles of *Cottus gobio* kidney (Bucher and Hofer, 1993), and in the euryhaline *Oreochromis niloticus* (Kamunde and Kisia, 1994).

A closed relationship could be assessed between the size of glomeruli and the habitat of different fish species. Marine teleosts have scarce small corpuscles with a 33-94 μm diameter (Marshall and Smith 1930, in De Ruiter 1980), which even are lacking in antartic fishes, such as notothenioids (Eastman and De Vries, 1986). In contrast, freshwater teleosts present abundant and bigger corpuscles. The freshwater species *Tinca tinca* exhibits large, and well irrigated glomeruli, considered by Ditrich and Splechtna (1986) the "basic type", as in other freshwater species such as Salmo gairdneri and in the water turtle *Pseudemys scripta*.

Oligosarcus jenynsi and *R. sapo* glomeruli correspond to the "basic type", according to their size and morphology, with a well developed capillar net that efficiently functions as a "water gland", removing the excess of water taken from the environment (Ditrich and Splechtna, 1986).

The neck segment is the shortest of all segments in fish nephron, and presents low columnar cells in the "catfish" and in the characid, but they have no evidence of cilia, that do exist in some euryhaline fishes, where they colaborate to the movement of glomerular filtrate (Kamunde and Kisia, 1994).

In both species, the proximal segment contains columnar cells with a prominent brush border in the apical region, which shows a positive reaction for PAS. These observations have been reported by Grizzle and Rogers (1976) for the "channel catfish", by Bucher and Hofer (1993) for other freshwater teleosts, by Kamunde and Kisia (1994) for the euryhaline "tilapia", and by Díaz *et al.* (1996) for the "hake" and "white croaker".

The second proximal segment epithelium is acidophilic, with a brush border less prominent than the former, and weakly positive for PAS. These characteristics are similar to those of the salmonid "rainbow trout" (Yasutake and Wales, 1983). The intense acidophilia and the microvilli suggest an active transport of ions (Kamunde and Kisia, 1994).

According to different physiological studies, the distal portions of fish nephron are important places for the movement of water and ions, allowing the urine dilution.

As well as occurs in other freshwater teleosts, a fairly well - developed distal tubule was observed in *O. jenynsi* and *R. sapo*. Bucher and Hofer (1993) demonstrated the existence of a distal segment and a nephron structure typical of freshwater teleosts in *Cottus gobio* kidney, whilst Anderson and Mitchum (1974), and Kamunde and Kisia (1994) suggested that the distal tubule plays the role of conservating salts. Contrarily, this tubule is reduced or absent in marine teleosts, as recorded by Díaz *et al.* (1996) for *Merluccius hubbsi* and *Micropogonias furnieri*.

As in many teleosts (e.g. *Cyprinus carpio*, *Salmo gairdneri*, *Morone saxatilis* and *Ictalurus punctatus*), lymphocytes are detected among the epithelial cells of the renal tubules in *O. jenynsi* and *R. sapo*. Lymphocytes are the most important cells in immune response and are usually present under normal conditions. Yasutake and Wales (1983) suggested that the lymphocytes are wandering cells from the adjacent hematopoietic tissue, which migrate to the tubular lumen in considerable number.

The results obtained in the present study allow to conclude that no marked differences exist between the histological structure of the nephron of *Oligosarcus jenynsi* and *Rhamdia sapo*, and that the kidney of both species is adapted to get rid of the osmotic water loads, in order to avoid an excesive hydration.

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REFERENCES

ANDERSON, B.G. and MITCHUM, D.L.,1974. Atlas of trout histology. Wyoming Game and Fish Commission Bulletin 13.

BALM, P.H.M.; HAENEN, H.E.M.G. and WENDELAAR BONGA, S.E., 1995. Regulation of interrenal function in freshwater and sea water adapted tilapia (*Oreochromis mossambicus*). Fish Physiology and Biochemistry, 14 (1): 37-47.

BUCHER, F. and HOFER, R., 1993. Histological and enzyme histochemical changes in the kidney of male bullhead (*Cottus gobio*) during the spawning period. Journal of Fish Biology, 42: 403-409.

CAMPANA, M.A.; PANZERI, A.M.; ESCALANTE, A.H.; MORENO, V.J. and DULOUT, F.N., 2001. Micronucleus test in fish from a pampasic pond (Argentina): an estimation of the presence of genotoxic compounds. Journal of Environmental Pathology, Toxicology and Oncology, 20 (4): 325-331.

DE RUITER, A.H.H., 1980. Changes in glomerular structure after sexual maturation and seawater adaptation in males of the euryhaline teleost *Gasterosteus aculeatus L.* Cell. Tiss. Res., 206: 1-20.

DÍAZ, A.; DEVINCENTI, C.; RICCI, L. and GOLDEMBERG, A., 1996. Comparative histology and histochemistry of hake (*Merluccius hubbsi*) and white croaker (*Micropogonias furnieri*) kidney. J. Anim. Morphol. Physiol., 43 (1): 59-63.

DÍAZ, A. and GOLDEMBERG, A., 1998. Sistema excretor en peces, pp. 85-92. In: Temas de Histología y Embriología Animal, parte 3 (A.C. Nessi ed.). Inarbite Ediciones, Bs. As.

DITRICH, H. and SPLECHTNA, H., 1986. Functional aspects of renal glomeruli based on scanning electron microscopy of corrosion casts, with special emphasis on reptiles and birds. Scanning Electron Microscopy, 2: 591-597.

EASTMAN, J.T. and DE VRIES, A.L., 1986. Renal glomerular evolution in Antarctic notothenioid fishes. J. Fish Biol., 29: 649-662.

ESCALANTE, A.H.; PARADA, V. and SOLARI, L.C., 1998. Caracterización fitoplanctónica del ecosistema Laguna de Los Padres: estudio cualitativo preliminar. Resúmenes Segundas Jornadas Bonaerenses de Microbiología Clínica, Ambiental, Industrial y de Alimentos. Mar del Plata: 23 .

GONZÁLEZ SAGRARIO, M. de los A.; AIZPÚN de MORENO, J. E.; MORENO, V. J. and ESCALANTE, A. H. , 1998. Dynamics of organochlorine compounds in different trophic levels of Los Padres pond in Argentina. I . Pesticides. Environmental Sciences, 6 (3): 153-169.

GONZÁLEZ SAGRARIO, M. de los A.; MIGLIORANZA, K. S. B.; AIZPÚN de MORENO, J. E.; MORENO, V. J. and ESCALANTE, A. H., 2002. Dynamics of polychlorinated biphenyls (PCBs) in different trophic levels from a shallow lake in Argentina. Chemosphere, 48 (10): 1113-1122.

GRIZZLE, J. and ROGERS, W., 1976. Anatomy and Histology of the channel catfish. Auburn University, Agricultural Experiment Station. Auburn, Alabama,6: 35-39.

GROMAN, D., 1982. Histology of the striped bass. American Fisheries Society, Bethesda. Maryland. Monograph 3: 48-52.

HUMASON, G.L., 1962. Animal tissue techniques. W.H.Freeman and Company .Eds. San Francisco, 468 pp.

KAMUNDE, C.N. and KISIA, S.M., 1994. Fine structure of the nephron in the euryhaline teleost, *Oreochromis niloticus*. Acta Biologica Hungarica, 45 (1): 111-121.

MARTÍNEZ, M.M.; VEGA, L.; VASALLO, A. and MALIZIA, A., 1995. Mapa inventario fauna, 16-32 and 101-111 pp. In: Carta ambiental del partido de Gral. Pueyrredón, Tomo I, Coord.: L. Del Río, M.J. Bó, J. Martínez Arca, M.V. Bernasconi. Univ. Nac. de Mar del Plata. Municipalidad de Gral. Pueyrredón, 112 pp.

MARTOJA, R. and MARTOJA-PIERSON, M., 1970. Técnicas de histología animal. Ed. Toray, Masson S.A. Barcelona, 350 pp.

MIGLIORANZA, K.S.B.; AIZPÚN de MORENO, J.E.; MORENO, V.J.; OSTERRIETH, M.L. and ESCALANTE, A.H., 1999. Fate of organochlorine pesticides in soils and terrestrial biota of "Los Padres" pond watershed, Argentina. Environmental Pollution, 105: 91-99.

MIGLIORANZA, K.S.B.; GONZÁLEZ SAGRARIO, M. de los A.; AIZPÚN de MORENO, J.E.; MORENO, V.J.; ESCALANTE, A.H. and OSTERRIETH, M.L., 2002. Agricultural soil as a potential source of input of organochlorine pesticides into a nearby pond. Environ. Sci. & Pollut. Res., 9 (4): 250-256.

OGURI, M., 1987. On the kidney of the porcupine fish as glomerular type. Nippon Suisan Gakkaishi, 53 (1): 57-58.

POZZOBON, M.V. and TELL, G., 1995. Estructura y dinámica de la comunidad perifítica sobre *Ricciocarpus natans* (Hepaticae) de la Laguna de Los Padres (Buenos Aires, Argentina). Bol. Soc. Argent. Bot., 30 (3-4): 199-208.

RINGUELET, R.A., 1975. Zoogeografía y ecología de los peces de aguas continentales de la Argentina y consideraciones sobre las áreas ictiológicas de América del Sur. Ecosur (Argentina),2 (3): 1-122.

RINGUELET, R.A.; ARÁMBURU, R.H. and ALONSO de ARÁMBURU, A, 1967. Los peces argentinos de agua dulce. Comisión de Investigación Científica, La Plata, 602 pp.

SOKAL, R.R. and ROHLF, F.J., 1979. Biometría. Principios y métodos estadísticos en la investigación biológica. Ed. H. Blume, Madrid, 832 pp.

YASUTAKE, W. and WALES, J., 1983. Microscopic anatomy of salmonids: An atlas. U.D. Department of the Interior. Fish and Wildlife Service. Publ. 150, Washington, 9: 97-103.