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COMPARATIVE CHARACTERISTICS OF THE ULTRASTRUCTURE OF NEPHRON CELLS IN SOME SPECIES OF PELAGIC, EPIBENTHIC, AND DEMERSAL FISH (THE KARANTINNAYA BAY, THE BLACK SEA)

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The ultrastructure of the main sections of the mesonephros nephron in Black Sea teleost fish is studied. The species investigated are as follows: pelagic *Trachurus mediterraneus* (Steindachner, 1868) and *Chelon auratus* (Risso, 1810); epibenthic *Diplodus annularis* (Linnaeus, 1758) and *Spicara flexuosa* Rafinesque, 1810; and demersal *Scorpaena porcus* Linnaeus, 1758, *Gobius niger* Linnaeus, 1758, and *Mullus barbatus ponticus* Essipov, 1927. It is shown that in Black Sea fish, which inhabit different depths and are under different conditions of environmental osmotic pressure, nephrons at the tissue level of organization have a single structure and form glomerular kidneys. Fish adaptability to the habitat at certain depths is primarily manifested in an increase in the number and size of mitochondria of all types of nephron epithelial cells. A decrease in the renal corpuscles area, the length of podocytes, and height of tubular epithelial cells, as well as the brush border length of type I proximal tubules is also recorded. Nephron cytological peculiarities of pelagic, epibenthic, and demersal fish characterize a high adaptive capacity of the mesonephros cellular structures.

Keywords: teleost fish, pelagic fish, epibenthic fish, demersal fish, kidney, nephron, ultrastructure, Black Sea

Determining the mechanisms of fish adaptation to diverse biotic and abiotic factors does not seem possible without a comprehensive study of the structure of various organs, tissues, and especially cells. Fish kidneys serve as a leading effector component of the physiological system of water-salt metabolism, due to which fish have acquired a certain habitat independence and inhabited both seawater and freshwater (Natochin, 1976, 2002 ; Wood et al., 2020). As known, the structure and function of the kidneys of freshwater and marine fish are determined by the peculiarities of their phylogenetic development and ecology. Freshwater teleost fish have a well-developed glomerular kidney, that excretes excess water and reabsorbs filtered ions. On the contrary, marine teleost fish have to save water and excrete excess salts; therefore, glomeruli are reduced in the kidneys of several species, up to their complete disappearance (Natochin, 1976, 2002 ; Erisson & Olsen, 1968 ; Ericsson & Olsen, 1970 ; Marshall, 1930).

Currently, special attention is given to the study of fish adaptations to a complex of environmental factors at the cellular level, since fish evolution is closely related to a high degree of specialization and adaptive plasticity of this level of organization of living matter. To develop a general pattern of kidneys functioning of teleost fish inhabiting water of various salinity, information is required on peculiarities of fine structures forming the nephron. Unfortunately, works focused on the study of ultrastructure of marine fish kidneys are still fragmentary. There is no data on the correlation between the nephron fine structure and the lifestyle of marine fish inhabiting different depths within the same water area characterized by different hydrochemical indicators, *inter alia* salinity (Kuftarkova et al., 2008). The present work is aimed at revealing common and specific features of the submicroscopic structure of the nephron in pelagic, epibenthic, and demersal teleost fish of the Black Sea.

MATERIAL AND METHODS

The ultrastructure of the mesonephros nephrons was studied in 7 species of Black Sea teleost fish. Pelagic species investigated are Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner, 1868) (7 ind., (10.1 ± 0.20) cm, (1.6 ± 0.80) g) and golden grey mullet *Chelon auratus* (Risso, 1810) (5 ind., (15.4 ± 7.7) cm, (49.0 ± 7.26) g). Epibenthic species are annular sea bream *Diplodus annularis* (Linnaeus, 1758) (10 ind., (5.60 ± 0.20) cm, (5.90 ± 0.50) g) and picarel *Spicara flexuosa* Rafinesque, 1810 (17 ind., (9.95 ± 0.19) cm, (18.3 ± 1.17) g). Demersal species are black scorpionfish *Scorpaena porcus* Linnaeus, 1758 (10 ind., (11.9 ± 0.46) cm, (67.4 ± 7.56) g), black goby *Gobius niger* Linnaeus, 1758 (3 ind., (8.70 ± 0.31) cm, (18.2 ± 0.92) g), and red mullet *Mullus barbatus ponticus* Essipov, 1927 (12 ind., (12.2 ± 0.44) cm, (62.6 ± 7.17) g).

Samples were taken in the summer-autumn period in the Karantinnaya Bay (the Black Sea, Sevastopol), which is characterized by the difference in temperature and salinity between surface and bottom. The temperature difference reaches 13.39 °C in summer. During this period, an inflow to the bottom layer of cooled and more saline (18.24 ‰) deep water with a reduced dissolved oxygen content (89 %) and pH value of 8.15 is clearly observed, while in the surface layer, salinity value averages 17.12 ‰, dissolved oxygen content – 96 %, and pH value – 8.24 (Kuftarkova et al., 2008).

Fish were caught with traps and transported to the laboratory to determine their size and weight characteristics. Then, kidneys were removed, and kidney pieces were dissected with a scalpel from the middle area of the mesonephros for electron microscopy. Samples were fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer and treated by an electron microscopy standard technique (Timakova et al., 2014). Ultrathin sections were prepared using a Leica EM UC7 microtome, contrasted with uranyl acetate and lead citrate, and examined under a JEM-1011 electron microscope. Measurements were carried out by digital photographs; data obtained were statistically processed using Microsoft Excel and Statistica 10 software.

During statistical processing, mean values and their standard errors ($M \pm m$) were calculated. The data analysis on outliers was performed *prior* to statistical analysis. Compliance with the normal distribution was assessed by the Shapiro – Wilk test (W). To determine the statistical significance of the difference in sample mean values, the Student's t-test was used. To assess the difference in sample mean values, multiple pairwise post hoc comparisons were performed, by the least significant difference test (LSD-test). If the distribution deviated from the normal, the Kruskal – Wallis test was used. In this case, the difference in sample mean values was assessed by multiple pairwise post hoc comparisons by the Dunnett's test. As a critical significance level, $p \leq 0.05$ was taken.

RESULTS

The renal corpuscle is the beginning of the nephron in all the species studied and is built according to a single principle. The wall of the renal corpuscle consists of two layers, parietal and visceral, very tightly contiguous to each other. Between the layers, there is a cavity, 2.03–2.45 μm thick (Fig. 1a, Table 1). The outer layer of the capsule is formed by a single-layer squamous epithelium located on the 0.66–0.77-μm thick basement membrane (Table 1). The inner layer of the capsule is formed by podocytes very tightly contiguous to each other (Fig. 1a). The podocyte body is oval; it is elongated along the nuclear membrane (Fig. 1b). The largest cells were identified on sections of renal corpuscles in pelagic species, *T. mediterraneus* and *Ch. auratus*. The length of T. *mediterraneus* podocytes significantly exceeds this indicator in epibenthic and demersal fish, while the length of *Ch. auratus* podocytes significantly exceeds this indicator in epibenthic *D. annularis*. The podocyte nucleus is rounded and occupies most of the cells; the length of the nuclei varies 2.82 to 4.34 μm, and the width varies 1.41 to 2.15 μm (Table 1). Heterochromatin is clumpy; it is concentrated mainly on the nucleus periphery. Dense cytoplasm of most cells contains two large mitochondria (Fig. 1b). On sections of renal corpuscles, a small number of capillary loops is observed (Fig. 1a).

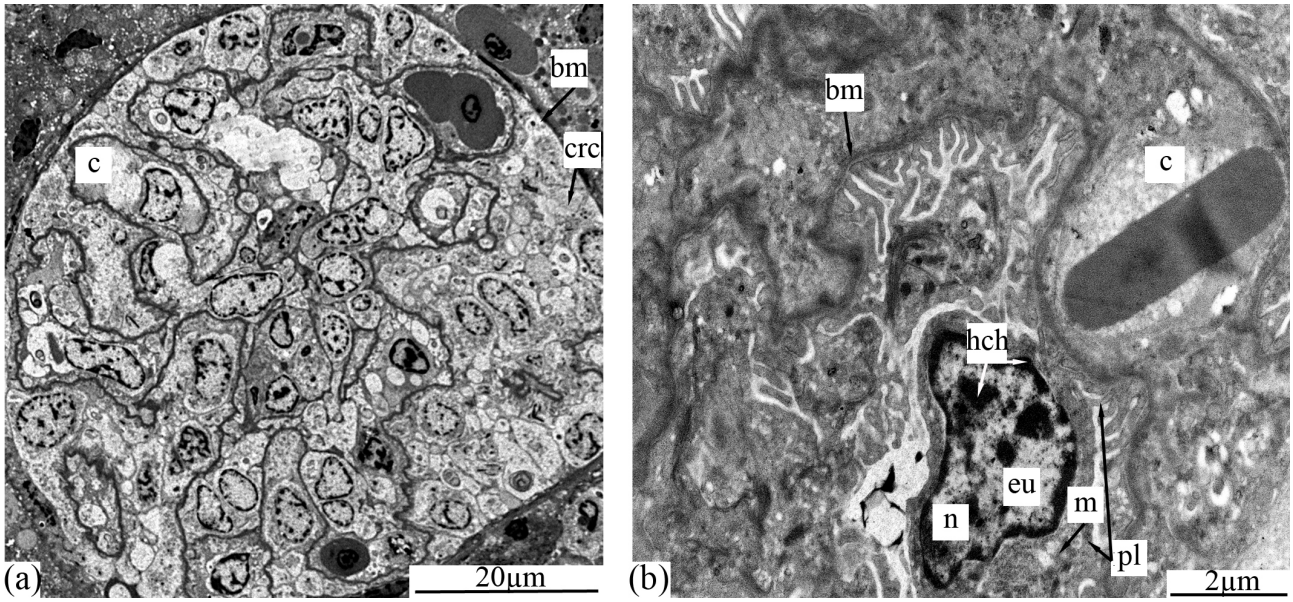

Fig. 1. Ultrastructure of the nephron: a – renal corpuscle in *Scorpaena porcus*; b – podocyte of the renal corpuscle in *Spicara flexuosa*. Bm – basement membrane of the parietal layer; hch – heterochromatin; c – capillary; m – mitochondrion; pf – podocyte feet; crc – cavity of renal corpuscle; eu – euchromatin; n – nucleus

The proximal tubule epithelial cells are built according to the plan typical for the cells of this nephron area (Figs 2a, 3a). They differ from the distal tubule cells by the presence of a brush border, which is the tallest in the initial area and gradually decreases as approaching the distal tubule epithelial cells.

Analysis of the ultrastructure of the cells lining this area of the tubule showed as follows: epithelial cells can be divided into 2 types (Figs 2a, 3a).

Table 1. Morphometric indicators of the renal corpuscle structures, μm

Species	Renal corpuscle diameter, $n = 10$	Renal corpuscle cavity, $n = 10$	Basement membrane thickness, $n = 20$	Podocyte	
				Cell, $n = 10$	Nucleus, $n = 20$
Trachurus mediterraneus	49.2 ± 3.60	2.08 ± 0.10	0.77 ± 0.05	$5.09 \pm 0.24^{1,2,3,4} \times 2.97 \pm 0.40$	$4.34 \pm 0.32 \times 2.15 \pm 0.30$
Chelon auratus	$49.0 \pm 0.41^{1,2,3,4,5}$	2.16 ± 0.07	0.73 ± 0.07	$4.76 \pm 0.34^{5} \times 2.86 \pm 0.19$	$3.60 \pm 0.16 \times 2.07 \pm 0.21$
Diplodus annularis	47.4 ± 0.48^{1}	2.03 ± 0.09	0.71 ± 0.05	$4.65 \pm 0.09^{5} \times 2.34 \pm 0.27$	$2.77 \pm 0.26 \times 1.41 \pm 0.21$
Spicara flexuosa	47.1 ± 0.66^{2}	2.08 ± 0.11	0.66 ± 0.06	$3.59 \pm 0.14^{1} \times 2.72 \pm 0.25$	$2.82 \pm 0.33 \times 1.92 \pm 0.12$
Gobius niger	47.0 ± 0.35^{3}	2.45 ± 0.22	0.67 ± 0.02	$4.19 \pm 0.34^{2} \times 2.49 \pm 0.29$	$3.11 \pm 0.44 \times 1.56 \pm 0.24$
Mullus barbatus ponticus	46.3 ± 0.17^{4}	2.42 ± 0.16	0.69 ± 0.01	$4.42 \pm 0.37^{3} \times 2.40 \pm 0.25$	$3.30 \pm 0.41 \times 1.57 \pm 0.20$
Scorpaena porcus	46.9 ± 0.82^{5}	2.33 ± 0.23	0.66 ± 0.03	$4.12 \pm 0.42^{4} \times 2.58 \pm 0.37$	$3.11 \pm 0.36 \times 1.81 \pm 0.28$

Note: hereinafter in the tables, the same numerical indices in different columns denote statistically significant differences between indicators, $p \leq 0.05$.

Type I epithelial cells form the beginning of the proximal tubule. These are elongated, pyramidal cells tightly contiguous to each other (Fig. 2a). The tallest epithelial cells were identified in pelagic species (*Ch. auratus*), and the shortest ones were recorded in demersal species (*S. porcus*). As revealed, the cells in *T. mediterraneus* and *Ch. auratus* are significantly longer than cells in other species studied (Table 2). The nuclei of epithelial cells are rounded; nuclei sizes differ insignificantly between the species (Table 2). The nuclei are located in the basal part of cells; there is little heterochromatin, and it is located mainly along the nuclear membrane, between nuclear pores (Fig. 1a). The cytoplasm contains a large number of large mitochondria located along the longitudinal axis of the cells (Fig. 2b). As established, the number and size of mitochondria on sections of epithelial cells increase in the series pelagic – epibenthic – demersal fish. Differences in the number and size of mitochondria between pelagic and demersal fish are significant (Table 2). Mitochondria in *D. annularis*, having an epibenthic lifestyle, are statistically significantly larger than those in demersal species, *G. niger* and *M. barbatus ponticus* (Table 2). Forming complex weaves, numerous folds of the smooth endoplasmic reticulum stretch from the basal part along the cells (Fig. 2b). In the basal part of cells, electron-transparent vesicles were revealed (Fig. 2b). In the apical part of cells, large electron-dense secretory granules were found, typical for this nephron area (Fig. 2a). The number of secretory granules varied insignificantly (Table 2). No correlation was revealed between the size of secretory granules and the lifestyle of the species studied. The largest granules were found on cell sections of *G. niger*, while the smallest secretory granules were recorded on cell sections of *M. barbatus ponticus* (Table 2). A well-developed endocytosis zone is located in the apical part of cells, at the edge of the brush border; it reaches the greatest length in *S. flexuosa*, while the lowest one – in *M. barbatus ponticus* (Table 2). This zone is characterized by the presence of a well-developed tubulovesicular system, which is formed by a large number of vesicles and single segments of tubular reticulum localized along the longitudinal axis of the cell (Fig. 2c).

Table 2. Morphometric indicators of type I epithelial cells of the proximal tubule, μm

Indicator	*Trachurus mediterraneus*	*Chelon auratus*	*Diplodus annularis*	*Spicara flexuosa*	*Gobius niger*	*Mullus barbatus ponticus*	*Scorpaena porcus*
Cell, $n = 10$	$13.4 \pm 0.51^{1,2,3,4,5} \times 10.5 \pm 0.70$	$13.8 \pm 0.62^{6,7,8,9,10} \times 9.15 \pm 0.44$	$12.5 \pm 0.38^{1,6} \times 9.57 \pm 0.42$	$12.0 \pm 0.20^{2,7} \times 9.57 \pm 0.30$	$11.7 \pm 0.29^{3,8} \times 9.32 \pm 0.28$	$11.3 \pm 0.18^{4,9} \times 8.40 \pm 0.88$	$11.0 \pm 0.39^{5,10} \times 9.04 \pm 0.62$
Nucleus, $n = 20$	$5.92 \pm 0.38 \times 4.40 \pm 0.39$	$4.27 \pm 0.20 \times 3.86 \pm 0.24$	$5.71 \pm 0.12 \times 4.02 \pm 0.26$	$4.78 \pm 0.42 \times 3.45 \pm 0.31$	$5.45 \pm 0.22 \times 4.80 \pm 0.28$	$4.13 \pm 0.11 \times 3.74 \pm 0.18$	$4.99 \pm 0.30 \times 3.53 \pm 0.41$
Mitochondrion, $n = 20$	$1.15 \pm 0.16 \times 0.59 \pm 0.04^{1,2,3,4}$	$1.02 \pm 0.08 \times 0.65 \pm 0.06^{5,6,7,8}$	$1.36 \pm 0.15 \times 0.79 \pm 0.05^{9,10}$	$1.82 \pm 0.62 \times 1.12 \pm 0.19^{1,5}$	$2.00 \pm 0.18 \times 1.26 \pm 0.11^{2,6,9}$	$2.10 \pm 0.21 \times 1.26 \pm 0.14^{3,7,10}$	$1.93 \pm 0.23 \times 1.49 \pm 0.14^{4,8}$
Number of mitochondria on cell section, $n = 20$	$48.7 \pm 7.28^{1,2}$	$45.3 \pm 3.48^{3,4}$	57.5 ± 3.19	52.0 ± 8.04	63.7 ± 4.83	$74.6 \pm 2.66^{1,3}$	$73.2 \pm 3.75^{2,4}$
Secretory granule, $n = 20$	$1.58 \pm 0.13 \times 1.35 \pm 0.13$	$0.68 \pm 0.06 \times 0.59 \pm 0.10$	$1.17 \pm 0.07 \times 0.93 \pm 0.06$	$1.08 \pm 0.20 \times 0.99 \pm 0.18$	$1.65 \pm 0.07 \times 1.47 \pm 0.13$	$0.63 \pm 0.03 \times 0.52 \pm 0.05$	$1.04 \pm 0.05 \times 0.95 \pm 0.05$
Number of secretory granules on cell section, $n = 20$	4.00 ± 0.45	4.00 ± 0.50	5.25 ± 1.31	5.29 ± 1.31	4.00 ± 0.49	3.20 ± 0.20	3.75 ± 0.68
Endocytosis zone, $n = 20$	3.81 ± 0.79	3.41 ± 0.21	3.25 ± 0.40	5.07 ± 0.25	4.65 ± 0.34	2.84 ± 0.32	4.64 ± 0.31
Brush border length, $n = 20$	$2.87 \pm 0.31^{1,2,3,4,5}$	2.69 ± 0.12	2.35 ± 0.14^{1}	1.99 ± 0.21^{2}	2.10 ± 0.18^{3}	1.95 ± 0.16^{4}	2.14 ± 0.12^{5}
Cilia, $n = 20$	0.23 ± 0.00	0.24 ± 0.00	0.22 ± 0.00	0.24 ± 0.00	0.23 ± 0.00	0.24 ± 0.00	0.24 ± 0.01
Microvilli, $n = 20$	0.32 ± 0.04	0.26 ± 0.01	0.40 ± 0.01	0.23 ± 0.03	0.31 ± 0.03	0.24 ± 0.02	0.23 ± 0.01

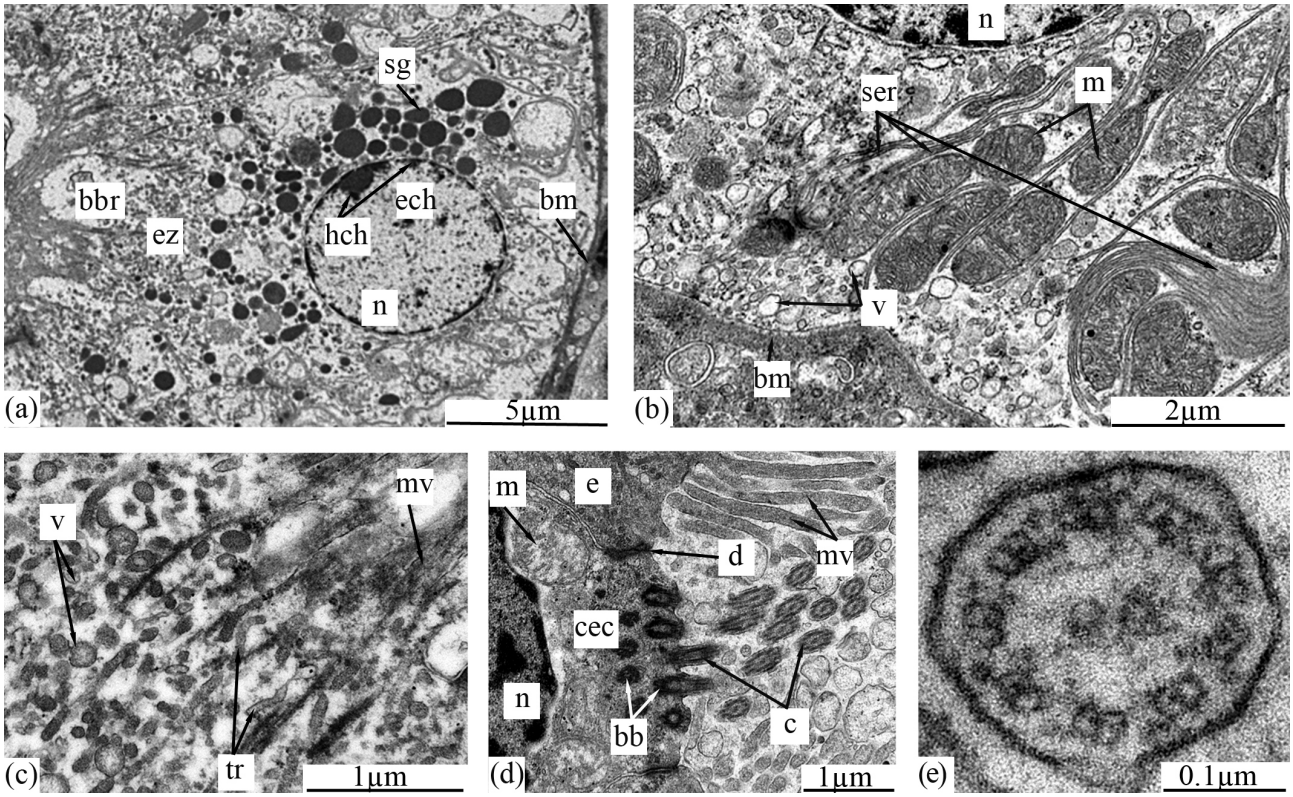

Fig. 2. Ultrastructure of type I proximal tubule epithelial cells: a – fragment of the proximal tubule with type I epithelial cells in *Chelon auratus*; b – basal part of type I epithelial cell in *Gobius niger*; c – endocytosis zone of *Mullus barbatus ponticus*; d – apical part of ciliated epithelial cell in *Diplodus annularis*; e – cross section of the cilium in *Diplodus annularis*. Bm – basement membrane; bb – basal body; v – vesicle; hch – heterochromatin; ser – smooth endoplasmic reticulum; d – desmosome; ez – endocytosis zone; m – mitochondrion; mv – microvilli; c – cilia; cec – ciliated epithelial cell; sg – secretory granules; bbr – brush border; tr – tubular reticulum; e – type I epithelial cell; ech – euchromatin; n – nucleus

The brush border is the tallest in the proximal tubules in pelagic fish: its length in *T. mediterraneus* is significantly larger than in epibenthic and demersal fish (Table 2). The brush border consists of a large number of cilia and microvilli facing the tubule lumen (Fig. 2d). As shown, the microvilli thickness differs in the species studied; thus, the indicator in *T. mediterraneus* significantly differs from that in *D. annularis*, *S. flexuosa*, and *S. porcus*. No correlation was revealed between the microvilli thickness and the lifestyle of the species (Table 2). Cilia are outgrowths of ciliated epithelial cells, which form the proximal tubule (Fig. 2d). The structure of ciliated epithelial cells is somewhat different from that of epithelial cells carrying microvilli on apical surface. The cytoplasm of ciliated cells is lighter, and there is no endocytosis zone; in the apical part of cells, large mitochondria are found located above cilia basal bodies (Fig. 2d). The structure of cilia is typical for these organelles, and they are formed by an axoneme; at the base of the cilium, there is a basal body (Fig. 2e).

Type II epithelial cells are structurally similar to type I cells, but are shorter (Fig. 3a, Table 3).

The shortest epithelial cells were recorded in the nephrons in *Ch. auratus*, and the tallest – in the nephrons in *S. porcus* (Table 3). Epithelial cells in *T. mediterraneus* were found to be significantly taller than those in *G. niger*, *M. barbatus ponticus*, and *S. porcus*. Epithelial cells in *Ch. auratus* were significantly taller than those in *T. mediterraneus* and in all epibenthic and demersal species studied (Table 3). The nuclei of epithelial cells are rounded, and they are located in the central area of the cells; nuclei sizes differ insignificantly between the species studied (Table 3, Fig. 3a). There is little heterochromatin,

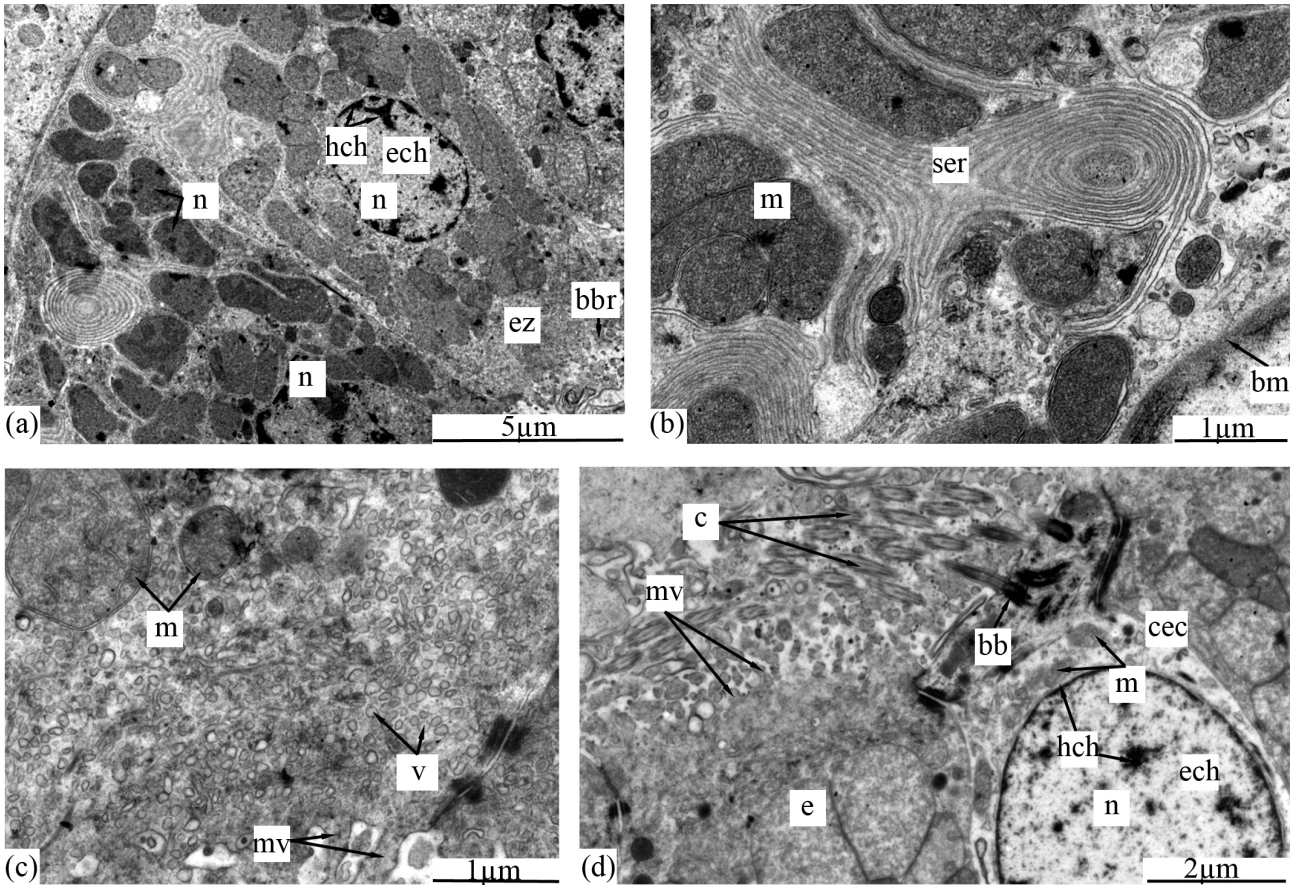

Fig. 3. Ultrastructure of type II proximal tubule epithelial cells: a – fragment of the proximal tubule with type II epithelial cells in *Mullus barbatus ponticus*; b – basal part of type II epithelial cell in *Gobius niger*; c – endocytosis zone of *Trachurus mediterraneus*; d – apical part of ciliated epithelial cell in *Trachurus mediterraneus*. Bm – basement membrane; bb – basal body; v – vesicle; hch – heterochromatin; ser – smooth endoplasmic reticulum; ez – endocytosis zone; m – mitochondrion; mv – microvilli; c – cilia; cec – ciliated epithelial cell; bbr – brush border; e – epithelial cell of the intermediate tubule; ch – euchromatin; n – nucleus

and it is located mainly along the nuclear membrane, between nuclear pores. The cytoplasm contains a large number of mitochondria; their mean number in type II epithelial cells exceeds that in type I cells. As revealed, the number of mitochondria on sections of epithelial cells in epibenthic and demersal fish significantly exceeds that on sections of epithelial cells in *T. mediterraneus* and *Ch. auratus* (Table 3). For type II epithelial cells, no pattern of changes in the size of mitochondria depending on lifestyle of the species studied was established. The largest mitochondria were registered on sections of *M. barbatus ponticus*, while the smallest ones – on sections of *T. mediterraneus*. The sizes of *S. flexuosa* and *M. barbatus ponticus* mitochondria were shown to significantly exceed those for *T. mediterraneus*. The sizes of *D. annularis* mitochondria significantly exceeded those for *S. flexuosa* and *M. barbatus ponticus* (Table 3). The folds of the smooth endoplasmic reticulum in the basal part of type II epithelial cells occupy a larger area compared to those of type I epithelial cells (Fig. 3b). A characteristic feature of type II epithelial cells is the absence of secretory granules in the cytoplasm (Fig. 3a). The endocytosis zone is less developed than in type I cells (Table 3), although a large number of vesicles are clearly visible (Fig. 3c). The endocytosis zone reaches the highest length values in *S. flexuosa*, and the lowest – in *T. mediterraneus* (Table 3). The brush border is shorter compared to that of type I cells (Table 3); it includes both cilia, which are the formation of ciliated epithelial cells, and microvilli (Fig. 3d). Microvilli of type II

cells are wider compared to microvilli of type I cells (Table 3). No correlation was found between the endocytosis zone length, brush border length, and microcilia thickness and the lifestyle of the species studied.

The intermediate tubule epithelial cells were found in the nephron in *Ch. auratus* and *S. porcus* (Fig. 4a, Table 4). The structure of these epithelial cells is the most different from the structure of the cell types considered above. These are the lowest cells, with nuclei in the central part. The epithelial cells of the nephron in *Ch. auratus* are significantly taller than those of the nephron in *S. porcus*. The nuclei structure of these epithelial cells is similar to that of types I and II cells (Fig. 4a).

On cell sections, a less developed, than in type I and II epithelial cells, system of tubules of the smooth endoplasmic reticulum was registered, which surrounds electron-dense mitochondria (Fig. 3b). *S. porcus* mitochondria are larger than those in *Ch. auratus*. The number of mitochondria on cell sections of *S. porcus* is more than 1.5 times higher than that on cell sections of *Ch. auratus*; the differences are significant. There is no formed endocytosis zone. Single microvilli are located on cell apical surface (Fig. 4a). The microvilli length of the intermediate tubule cells is 2 times less than that of the microvilli of types I and II epithelial cells. *Ch. auratus* microvilli are significantly taller than those of *S. porcus* (Table 4). In the intermediate tubule structure, there are no ciliated epithelial cells.

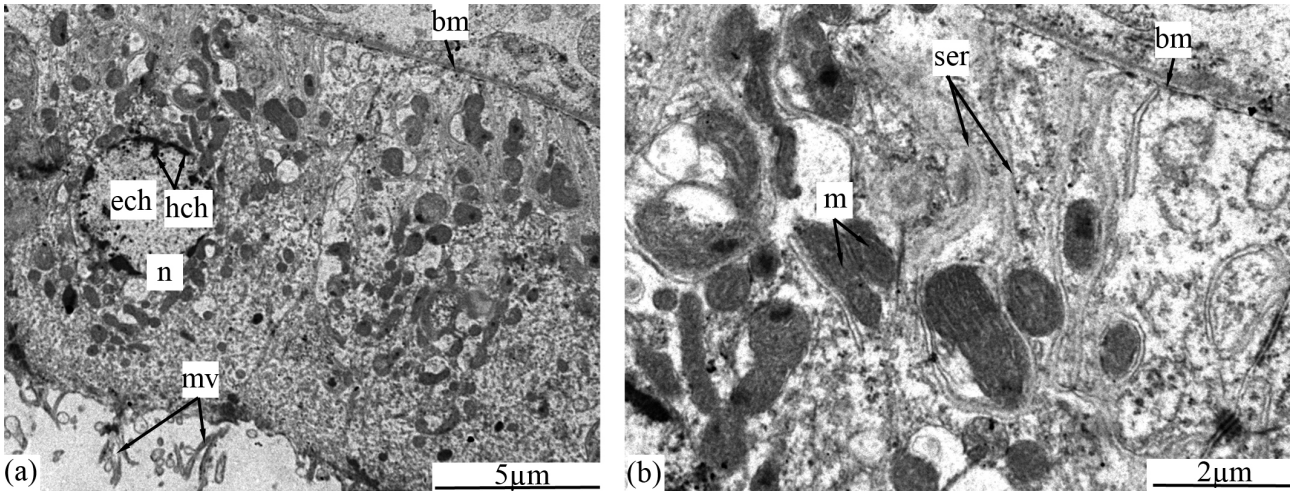

Fig. 4. Ultrastructure of the intermediate tubule epithelial cells: a – fragment of the intermediate tubule in *Chelon auratus*; b – basal part of the intermediate tubule epithelial cell in *Chelon auratus*. Bm – basement membrane; hch – heterochromatin; ser – smooth endoplasmic reticulum; m – mitochondrion; mv – microvilli; ech – euchromatin; n – nucleus

The distal tubule is formed by cells, that are tall and very wide at the base (Fig. 5a, Table 5).

The tallest epithelial cells of this part of the tubule were found in the nephron in *D. annularis*, and the shortest ones – in the nephron in *M. barbatus ponticus*. It was shown as follows: the height of epithelial cells decreases in the series pelagic – epibenthic – demersal fish. When comparing the sizes of epithelial cells in pelagic and demersal fish, it was revealed that the epithelial cells in *T. mediterraneus* were significantly taller than those in *G. niger* and *M. barbatus ponticus*. Epithelial cells in *D. annularis* were significantly taller than those in *G. niger*, *M. barbatus ponticus*, and *S. porcus*. Comparing the sizes of epithelial cells in epibenthic and demersal fish, it was recorded as follows: *Ch. auratus* cells were statistically significantly taller than those of demersal fish, and *S. flexuosa* epithelial cells were significantly taller than those of *M. barbatus ponticus* (Table 5).

Table 3. Morphometric indicators of type II epithelial cells of the proximal tubule, μm

Indicator	*Trachurus mediterraneus*	*Chelon auratus*	*Diplodus annularis*	*Spicara flexuosa*	*Gobius niger*	*Mullus barbatus ponticus*	*Scorpaena porcus*
Cell, $n = 10$	$11.2 \pm 0.24^{1,2,3,4} \times 9.74 \pm 1.50$	$12.8 \pm 0.37^{1,5,6,7,8,9} \times 9.74 \pm 0.19$	$11.2 \pm 0.78^{5} \times 7.55 \pm 0.61$	$10.2 \pm 1.10^{6} \times 7.85 \pm 0.60$	$9.76 \pm 0.56^{2,7} \times 8.38 \pm 0.75$	$9.55 \pm 0.14^{3,8} \times 8.46 \pm 0.22$	$9.16 \pm 0.18^{4,9} \times 7.32 \pm 0.42$
Nucleus, $n = 20$	$4.54 \pm 0.24 \times 3.29 \pm 0.12$	$4.43 \pm 0.05 \times 3.70 \pm 0.16$	$3.33 \pm 0.12 \times 3.01 \pm 0.02$	$4.98 \pm 0.43 \times 3.94 \pm 0.34$	$4.70 \pm 0.16 \times 3.56 \pm 0.31$	$4.52 \pm 0.13 \times 3.45 \pm 0.19$	$5.44 \pm 0.21 \times 4.99 \pm 0.04$
Mitochondrion, $n = 20$	$1.19 \pm 0.21 \times 0.73 \pm 0.10^{1,2}$	$1.39 \pm 0.10 \times 1.19 \pm 0.11$	$1.26 \pm 0.21 \times 0.83 \pm 0.09^{3,4}$	$1.57 \pm 0.07 \times 1.17 \pm 0.05^{1,3}$	$1.61 \pm 0.13 \times 0.80 \pm 0.03$	$1.72 \pm 0.21 \times 1.23 \pm 0.19^{2,4}$	$2.74 \pm 0.74 \times 0.89 \pm 0.05$
Number of mitochondria on cell section, $n = 20$	$50.0 \pm 3.55^{1,2,3,4,5}$	$48.0 \pm 4.24^{6,7,8,9,10}$	$75.0 \pm 3.77^{1,6}$	$72.3 \pm 0.76^{2,7}$	$78.7 \pm 3.54^{3,8}$	$76.2 \pm 2.29^{4,9}$	$76.6 \pm 1.60^{5,10}$
Endocytosis zone, $n = 20$	$1.24 \pm 0.31^{1,2}$	2.01 ± 0.23	1.83 ± 0.18	4.19 ± 0.30^{1}	1.41 ± 0.20	2.01 ± 0.15	2.64 ± 0.12^{2}
Brush border length, $n = 20$	1.58 ± 0.19	1.81 ± 0.27	1.45 ± 0.15	1.96 ± 0.40	1.58 ± 0.22	1.39 ± 0.03	1.57 ± 0.11
Cilia, $n = 20$	0.23 ± 0.00	0.24 ± 0.00	0.22 ± 0.00	0.24 ± 0.00	0.23 ± 0.00	0.24 ± 0.01	0.24 ± 0.00
Microvilli, $n = 20$	0.33 ± 0.02	0.27 ± 0.02	0.45 ± 0.04	0.26 ± 0.03	0.37 ± 0.03	0.27 ± 0.02	0.48 ± 0.02

Table 4. Morphometric indicators of the intermediate tubule epithelial cells, μm

Indicator	*Chelon auratus*	*Scorpaena porcus*
Cell, $n = 10$	$11.8 \pm 0.26 \times 9.32 \pm 0.22$	$9.07 \pm 0.22^{*} \times 7.50 \pm 0.23$
Nucleus, $n = 20$	$4.97 \pm 0.30 \times 4.61 \pm 0.16$	$4.96 \pm 0.61 \times 4.60 \pm 0.33$
Mitochondrion, $n = 20$	$1.20 \pm 0.10 \times 1.03 \pm 0.05$	$1.54 \pm 0.09 \times 1.24 \pm 0.08^{*}$
Number of mitochondria on cell section, $n = 20$	36.6 ± 3.65	$63.4 \pm 2.77^{*}$
Microvilli length, $n = 20$	0.85 ± 0.17	$0.50 \pm 0.07^{*}$
Microvilli, $n = 20$	0.33 ± 0.04	0.29 ± 0.01

Note: * – the differences between the indicators for *Chelon auratus* and *Scorpaena porcus* are statistically significant, $p \leq 0.05$.

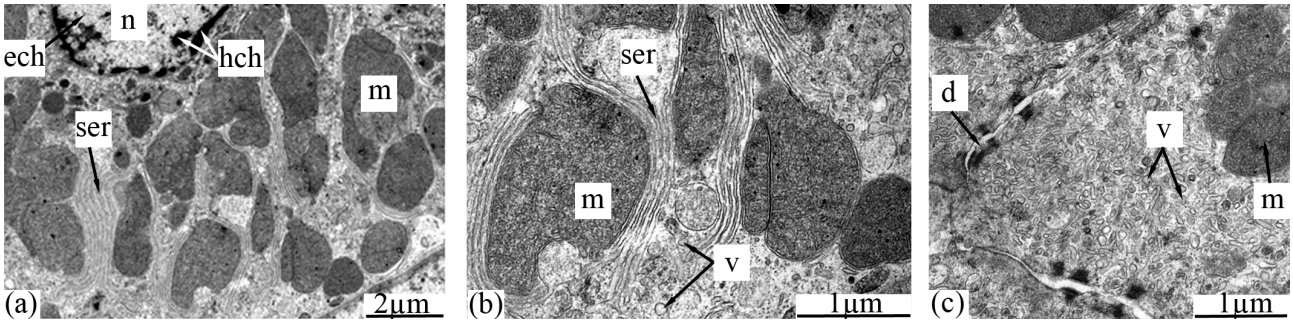

Fig. 5. Ultrastructure of the distal tubule epithelial cells: a – basal part of the distal tubule epithelial cells in *Mullus barbatus ponticus*; b – cytoplasm of the distal tubule epithelial cells in *Mullus barbatus ponticus*; c – lobe-like outgrowths of the distal tubule epithelial cells in *Mullus barbatus ponticus*. V – vesicles; hch – heterochromatin; ser – smooth endoplasmic reticulum; d – desmosome; m – mitochondrion; mv – microvilli; ech – euchromatin; n – nucleus

Table 5. Morphometric indicators of the distal tubule epithelial cells, μm

Indicator	Cell, $n = 10$	Nucleus, $n = 20$	Mitochondrion, $n = 20$	Number of mitochondria on cell section, $n = 20$
Trachurus mediterraneus	$14.9 \pm 0.19^{1,2} \times 9.80 \pm 0.94$	$6.33 \pm 0.22 \times 4.90 \pm 0.16$	$1.48 \pm 0.16 \times 0.62 \pm 0.12^{1,2}$	$49.6 \pm 4.59^{1,2,3,4,5,6}$
Chelon auratus	$15.3 \pm 0.64^{3,4,5} \times 15.2 \pm 1.63$	$6.16 \pm 0.35 \times 4.92 \pm 0.66$	$1.49 \pm 0.21 \times 0.74 \pm 0.05$	$47.2 \pm 5.20^{1,7,8}$
Diplodus annularis	$14.5 \pm 0.76^{6,7,8} \times 12.8 \pm 1.38$	$5.38 \pm 0.18 \times 4.88 \pm 0.24$	$1.83 \pm 0.07 \times 0.92 \pm 0.07^{3,4}$	$77.2 \pm 4.07^{2,7,9,10,11}$
Spicara flexuosa	$13.4 \pm 0.71^{9} \times 11.0 \pm 1.71$	$5.34 \pm 0.49 \times 4.93 \pm 0.57$	$1.55 \pm 0.16 \times 0.81 \pm 0.16^{5,6}$	$63.6 \pm 2.28^{3,8}$
Gobius niger	$13.2 \pm 0.15^{1,3,6} \times 9.63 \pm 0.34$	$5.82 \pm 0.33 \times 4.57 \pm 0.26$	$2.14 \pm 0.10 \times 1.28 \pm 0.06$	$84.4 \pm 1.33^{4,9}$
Mullus barbatus ponticus	$12.5 \pm 0.06^{2,4,7,9} \times 9.11 \pm 0.87$	$5.47 \pm 0.93 \times 3.50 \pm 0.18$	$1.99 \pm 0.12 \times 1.22 \pm 0.11^{1,3,5}$	$77.3 \pm 2.98^{5,10}$
Scorpaena porcus	$13.1 \pm 0.27^{5,8} \times 9.50 \pm 0.19$	$4.54 \pm 0.35 \times 3.60 \pm 0.21$	$2.01 \pm 0.18 \times 1.22 \pm 0.11^{2,4,6}$	$84.2 \pm 1.34^{6,11}$

The nuclei of most cells occupy a central position; sometimes, they are displaced towards the basal part. There is little heterochromatin, and it is concentrated mainly on the nucleus periphery, between nuclear pores. In cytoplasm, large electron-dense mitochondria were found, which were less ordered than mitochondria of type I and II epithelial cells. Mitochondria are surrounded by a tubular system of the smooth endoplasmic reticulum, which is developed similarly to the system of type I proximal tubule epithelial cells (Fig. 5a, b). The number and size of mitochondria increase in the series pelagic – epibenthic – demersal fish. As revealed, the number of mitochondria on cell sections of *T. mediterraneus* was statistically significantly smaller than in other species studied. The number of mitochondria on cell sections of *D. annularis* was statistically significantly smaller than that of *Ch. auratus* and *S. flexuosa*. The number of mitochondria on cell sections of *Ch. auratus* was significantly smaller than in the epibenthic species (Table 5). It was revealed that mitochondria on cell sections of *M. barbatus ponticus* and *S. porcus* were statistically significantly larger compared to those in *T. mediterraneus* and epibenthic fish (Table 5). There is no endocytosis zone. A characteristic feature of this type of cells

is a large number of vesicles localized in the apical part of cells, which, in turn, forms lobe-like cytoplasmic outgrowths facing the tubule lumen. The largest number of vesicles, that fill the entire cytoplasm of lobe-like outgrowths, was recorded in demersal fish (Fig. 5c).

DISCUSSION

Cytological analysis of the mesonephros of pelagic, epibenthic, and demersal fish of the Black Sea, with the mean salinity of surface layers of 17.58–18.09 ‰ and of deeper layers of 22.33 ‰ (Ivanov & Belokopytov, 2011), has shown as follows: the nephrons of the trunk kidney of the species studied have a single structure. At the same time, the analysis of the results obtained unambiguously indicates several peculiarities of the nephron cell ultrastructure in the species, which depend on the confinement to a certain depth and salinity.

All kidneys are glomerular; the nephron includes both proximal and distal tubules. For all the species, a similar change in the length of epithelial cells, endocytosis zone, brush border, and microvilli diameter from proximal to distal nephron section is shown. Kidneys of marine teleost fish are known to be of two types, glomerular and aglomerular. Aglomerular kidneys were described for demersal ambush predators, angler *Lophius piscatorius* and oyster toadfish *Opsanus tau*, which inhabit ocean waters, with the mean annual salinity of about 35 ‰ at depth reaching 200 m, as well as for *Nerophis ophidian* inhabiting the Atlantic Ocean, depth of down to 30 m (Erisson & Olsen, 1968 ; Ericsson & Olsen, 1970 ; Marshall, 1930). Well-developed renal corpuscles were described for many species of freshwater and anadromous fish, as well as euryhaline species *Sparus auratus*, *Trachurus mediterraneus*, and *Diplodus annularis*, with a range mainly confined to the Black Sea (Flerova, 2012 ; Flerova et al., 2020 ; Zuasti & Agulleiro, 1983). Based on literature data and on our results, we can assume that renal corpuscles are characteristic of the nephrons in all teleost fish, inhabiting seawater with salinity up to 22 ‰, regardless of their lifestyle. Previously, a correlation between the salinity of the habitat and the level of glomeruli development was shown (Lozovik, 1963 ; Oğuz, 2015). Moreover, it was established that the diameter of the renal corpuscles and the body size of podocytes (the cells involved in the formation of the filtration barrier of the kidney) are larger in freshwater fish than in marine fish. This is primarily due to the fact that the kidneys of freshwater teleost fish filter larger volumes of fluid than the kidneys of marine fish (Flerova, 2012). Differences in the diameter of the renal corpuscle and the length of podocytes for pelagic, epibenthic, and demersal fish are likely to be related to the regulation of water-salt metabolism, when inhabiting various depths, with different salinity and water column pressure.

The proximal tubule turned out to be the most differentiated; it is formed by two types of epithelial cells, differing in their morphology. Such a structural organization of the proximal tubule is conservative for teleost fish. Thus, two types of epithelial cells were previously described for species of the orders Salmoniformes, Cypriniformes, and Perciformes inhabiting freshwater and seawater and performing anadromous migrations (Flerova, 2012 ; Flerova et al., 2020 ; Anderson & Loewen, 1975 ; Maksimovich et al., 2000 ; Ojeda et al., 2006). It is known that the brush border of the proximal tubules regulates the rate of active fluid transport (Natochin, 1976). In the proximal tubule, ciliated cells of all studied types were found, due to which both epithelial cells bearing microvilli on the apical surface and a brush border are formed; this demonstrates the similarity of its ultrastructure with the ultrastructure of freshwater fish (Flerova, 2012). No correlation was revealed between the frequency of occurrence of ciliated cells in the proximal tubules in the species studied and their lifestyle. Nevertheless, a shorter brush border of type I proximal tubules of the nephron in *T. mediterraneus* and *Ch. auratus* was recorded compared

to brush borders in other species studied; both this fact and a lower height of epithelial cells indicate a decrease in the volume of the glomerular filtrate coming from renal corpuscles of epibenthic and demersal fish compared to the volume of the glomerular filtrate of pelagic fish.

As known, the degree of development of a smooth endoplasmic reticulum and of associated mitochondria number directly depends on the intensity of the mechanisms of reabsorption and secretion of the proximal tubule epithelial cell ions (Natochin, 1976). For all the species studied, the higher degree of development of a smooth endoplasmic reticulum is revealed compared to similar organoid in Salmoniformes, Cypriniformes, and Perciformes freshwater fish, as well as Salmonidae smolts performing anadromous migrations (Flerova, 2012 ; Flerova et al., 2020, 2019). No correlation was found between the development of a smooth endoplasmic reticulum and the lifestyle. Pattern of an increase in the number and size of mitochondria with an increase in the depth and salinity of the habitat was noted. These structural changes indicate intensification of the work of pumps, which provide active transport of ions, under increased osmotic load; these pumps are located mainly in the basal part of cells (Natochin, 1976).

The next section of the tubule, which was found in *Ch. auratus* and *S. porcus* only, is formed by epithelial cells that have similar structure with the intermediate tubule epithelial cells in Salmoniformes, Cypriniformes, and Perciformes freshwater fish (Vinnichenko, 1980 ; Maksimovich et al., 2000). It can be assumed that the intermediate tubule is present in all the species studied, but since the epithelial cells of this section form a small segment of the nephron, it is extremely difficult to identify and describe them. Both the proximal tubule cells and intermediate tubule epithelial cells in *S. porcus* are characterized by lower height and shorter microvilli length, as well as by a significantly larger number of larger mitochondria compared to those in *Ch. auratus*. Previously, it was shown that the structure of epithelial cells indicates their similarity with the thin segment cells of the loop of Henle of nephrons in warm-blooded animals, with the main function being to transport water (Vinnichenko, 1980). The specialization of these cells in *Ch. auratus* and *S. porcus* allows suggesting that demersal fish, as compared to pelagic ones, have a more perfect system of anti-gradient processes, which, along with a larger flattening of cells, makes it possible to shorten the water path (Erisson & Olsen, 1968 ; Ojeda et al., 2006).

The distal tubule epithelial cells of the species studied have a similar structure with the cells, that were previously described for Salmoniformes, Cypriniformes, and Perciformes species of different ecological groups (Flerova, 2012 ; Flerova et al., 2020 ; Anderson & Loewen, 1975 ; Flerova et al., 2019 ; Maksimovich et al., 2000 ; Ojeda et al., 2006). As shown earlier, a large number of vesicles distributed throughout the cytoplasm of cells, a larger number of mitochondria on sections of the distal tubule epithelial cells as compared to a number in proximal tubules, and a more developed system of membranes of the smooth endoplasmic reticulum on sections of the distal tubule epithelial cells in marine fish compared to freshwater fish indicate the peculiarities of functioning of distal tubule related to the regulation of the excreted urine volume (Natochin, 1976). For the species studied, a pattern of an increase in the number and size of mitochondria and the number of vesicles with an increase in the depth and salinity of the habitat was registered. These structural changes can be cytological markers of an increase in salinity and water column pressure as well.

It should be noted as follows: the differences in the basement membrane thickness, width of the renal corpuscle cavity, length of the endocytosis zone, size of the nuclei of all types of cells, number and size of secretory granules in the cytoplasm of type I proximal tubules, and microvilli thickness were statistically insignificant and most likely related not to the systematic or ecological peculiarities of the species, but to the functioning of structures at a certain point in time.

Conclusion. Comparison of the ultrastructural peculiarities of the mesonephros in the Black Sea fish, which inhabit different depths and are under different conditions of environmental osmotic pressure, allows suggesting as follows: at the tissue level of organization, nephrons have a single structure and form glomerular kidneys. The confinement to the habitat at certain depths is primarily manifested in an increase in the number and size of mitochondria of all types of nephron epithelial cells. Smaller area of renal corpuscles, length of the podocytes, and height of tubular epithelial cells, as well as length of the brush border of type I proximal tubules are registered. Nephron cytological peculiarities of pelagic, epibenthic, and demersal fish characterize a high adaptive capacity of the mesonephros cellular structures.

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СРАВНИТЕЛЬНАЯ ХАРАКТЕРИСТИКА УЛЬТРАСТРУКТУРЫ КЛЕТОК НЕФРОНА НЕКОТОРЫХ ВИДОВ ПЕЛАГИЧЕСКИХ, ПРИДОННЫХ И ДОННЫХ РЫБ (БУХТА КАРАНТИННАЯ, ЧЁРНОЕ МОРЕ)

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Изучена ультраструктура основных отделов нефрона мезонефроса костистых рыб Чёрного моря (пелагических *Trachurus mediterraneus* (Steindachner, 1868) и *Chelon auratus* (Risso, 1810); придонных *Diplodus annularis* (Linnaeus, 1758) и *Spicara flexuosa* Rafinesque, 1810; донных *Scorpaena porcus* Linnaeus, 1758, *Gobius niger* Linnaeus, 1758 и *Mullus barbatus ponticus*

Essipov, 1927). Показано, что у рыб Чёрного моря, обитающих на разных глубинах и находящихся в различных условиях осмотической нагрузки среды, на тканевом уровне организации нефроны имеют единый план строения и формируют гломерулярные почки. Приспособленность рыб к обитанию на определённых глубинах в первую очередь проявляется в увеличении количества и размеров митохондрий всех типов эпителиальных клеток нефрона. Кроме того, отмечено уменьшение площади почечных телец, длины подоцитов и высоты эпителиоцитов канальцев, а также длины щёточной каёмки проксимальных канальцев I типа. Цитологические особенности нефрона пелагических, придонных и донных рыб характеризуют высокую адаптационную способность клеточных структур мезонефроса.

Ключевые слова: костистые рыбы, пелагические рыбы, придонные рыбы, донные рыбы, почка, нефрон, ультраструктура, Чёрное море