Morphological and physiological traits of the mesonephros in a freshwater fish, grayling \textit{Thymallus thymallus}

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The study presents new data on the structural and functional organization of the mesonephros of the grayling \textit{Thymallus thymallus} (Linnaeus, 1758). Adult grayling were sampled in the middle course of the Unya River, a tributary of the Pechora River (Komi Republic, Russia). The mesonephros of the grayling, as of other freshwater fishes, is composed by nephrons, blood vessels and hematopoietic tissue forming the renal interstice. In the interstice, cells with a radial vesicle array and chloride cells were discovered; the latter were mostly localized near the renal tubules. The degree of the interstice development in the mesonephros of the grayling was determined. New data on the ultrastructure of leukocytes, cells with a radial vesicle array, chloride cells, and nephron segments were obtained. A lack of mesangial cells, a small number of podocytes, and a thin basement membrane were observed on the sections of a renal corpuscle, being characteristic features of the ultrathin organization of the mesonephros in freshwater members of Salmoniformes and Esociformes. In the grayling’s nephrons, no neck segment was found, which was reported earlier for several species, including mammals. On the sections of proximal tubules, the ciliated cells were rare, and large amounts of the tubular-vesicular network in the zone of endocytosis of the type II epithelial cells were observed. On the sections of distal tubules, short blade-shaped cyttoplasmic processes, with large numbers of invaginations of cyttoplasmic membrane, were found. On the basis of the distinctive ultrastructure features mentioned above, the inference that grayling show the cytological markers of adaptation to euryhalinity was made. Thus, the results contribute to the knowledge of mesonephros development in fishes during their life history. From the species protection standpoint, our study provides baseline data on a WBC differential in the mesonephros as well as superoxide dismutase, catalase, glutathione S-transferase, and ethoxyresorufin-O-deethylase activities, which can be used in further studies addressing the health status of grayling populations.

Keywords: micrornatomy; ultrastructure; leukocytes; antioxidants; ethoxyresorufin-O-deethylase; kidney.

Introduction

In the course of evolution, a general mechanism of non-specific protection was developed. Immune, antioxidant, and monooxygenase systems are the most important components of this protection. These systems provide the interaction between an organism and its environment. From a comparative-evolutionary perspective, it is very important to study the structural and functional features of the mesonephros, which not only plays the main role in the osmotic regulation, but also contributes significantly to homeostasis at the level of the non-specific protection. In this regard, studies on the salmons, a unique family of anadromous and freshwater fishes having some relatively primitive morphological traits, are of current importance.

There are a number of studies on various aspects of mesonephros morphology and physiology of anadromous salmons in relation to the smoltification process (Langdon, 1985; Talbot et al., 1992; Boeuf, 1993; Mizuno et al., 2001; Björnsson & Bradley, 2007), while studies on morphological and physiological traits of the mesonephros in freshwater salmonids are few in number (Anderson & Loewen, 1975; Kocabatma & Ekingen, 1987; Katoh et al., 2008). The grayling, \textit{Thymallus thymallus} (Linnaeus, 1758) is a freshwater salmonid species. The grayling is a benthopelagic fish, which feeds on chironomid and simulid larvae, drifting macroinvertebrates, epheberopteran nymphs, fish larvae (Scott, 1985). It inhabits submontane reaches of rivers with a hard sand or stone bottom and well oxygenated, cold and fast-flowing water (Freyhof, 2011). The grayling is listed in the IUCN Red List with the Least Concern (LC) status. Grayling populations are threatened by overshing as well as by natural system modifications, such as dam construction and water management, and habitat pollution with domestic and urban wastewaters, industrial, military, agricultural, and forestry effluents (Nykanen et al., 2001; Freyhof, 2011).

The purpose of our study was to perform an integrated research on structural and functional organization of the mesonephros of grayling. The data obtained in this study are a valuable contribution to the comparative-evolutionary conception of the mesonephros ultrastructure in lower vertebrates. From a practical perspective, our results can be used in the aquatic biomonitoring as well as in ichthyopathological assessment procedures in grayling culture.
Materials and methods

The fish sampling performed within the study was conducted by the Fresh Water Fishes Laboratory (SevPINRO) in July 2012. Grayling was sampled in the middle course of the Unya River, a tributary of the Pechora River (Fig. 1). The Unya River flows 163 kilometers and has a catchment area of 2,890 square kilometers. The Unya River has a rocky stream bed, with rifts and rapids. By chemical composition, the river waters belong to the hydrocarbonate class; total dissolved solids concentrations vary from 200 to 500 mg/l. Fish sampling was carried out in the “Unyinsky” protected area, which is located in a buffer zone of the Pechora-Ilych Nature Reserve. Due to its remoteness and inaccessibility, the area does not experience any significant anthropogenic impact, and thus, the area was considered suitable for the baseline study of morphological and physiological traits of the mesonephros in grayling.

![Fig. 1. Sampling site (marked with a grey rectangle)](image)

Fish were collected using a minnow seine, 20 m long, with a 3 mm mesh in a bag. Ten sexually mature fish were used for the study. Each specimen was immobilized by a blow to the head, measured and weighed, the specimen was immobilized by a blow to the head, measured and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed, the fish was dissected, eviscerated and weighed. Each epithelial cell had an intensely stained oval or rounded nucleus, which in most cells, was located at the basal side. The distal tubule (OD 56.71 ± 2.59 μm) was also composed by epitheli...
The cytoplasm contained electron-dense phagosomes of various sizes (Table 2), which occupied almost the entire cell volume. The cytoplasm also contained mitochondria, short double membrane sheets of the rough endoplasmic reticulum, free ribosomes, lysosomes, and small light vesicles (Table 2, Fig. 2d). In some cells, a large number of the electron-dense pigment granules were found.

Fig. 2. Microanatomy of the mesonephros of grayling, *Thymallus thymallus*: a – mesonephros tissues; b – lymphocyte; c – plasma cell; d – macrophage; e – neutrophil; f – intra-cellular granule of a neutrophil; g – eosinophil; h – cell with a radial vesicle array; i – chloride cell; j, k – cytoplasm of a chloride cell; 1 – blood vessel; 2 – hematopoietic tissue; 3 – renal corpuscle; 4 – proximal tubule; 5 – distal tubule; 6 – nucleus; 7 – heterochromatin; 8 – euchromatin; 9 – mitochondrion; 10 – rough endoplasmic reticulum; 11 – phagosome; 12 – intra-cellular granule of a neutrophil; 13 – Golgi apparatus; 14 – intra-cellular granules of an eosinophil; 15 – radial vesicle array; 16 – tubular reticulum; 17 – secondary lysosome.
Fig. 3. Ultrastructure of nephron in mesonephros of grayling, *Thymallus thymallus*: a – renal corpuscle; b – podocyte; c – section of the proximal tubule; d – brush border; e – cytoplasm of the type I epithelial cell of the proximal tubule; f – basal part of the type I epithelial cell of the proximal tubule; g – section of the proximal tubule with a ciliated cell; h – basal part of the type II epithelial cell of the proximal tubule; i – zone of endocytosis; j – basal part of the distal tubule; k – apical part of the distal tubule; 1 – glomerular capillary; 2 – podocyte; 3 – epithelial cell of the parietal layer of the Bowman's capsule; 4 – Bowman's space; 5 – basement membrane of the parietal layer; 6 – basement membrane of the visceral layer; 7 – nucleus; 8 – pedicels of a podocyte; 9 – podocyte; 10 – tubular basement membrane; 11 – secretory granules; 12 – mitochondrion; 13 – smooth endoplasmic reticulum; 14 – tubular-vesicular network; 15 – brush border; 16 – brush border microvilli; 17 – basal body of a cilium; 18 – cilium; 19 – blade-shaped cytoplasmic processes.
Neutrophils were rounded (Fig. 2e). The shape of a nucleus varied from bean-like to segmental. Heterochromatin was segmented, localized mostly along the nucleus membrane. The cytoplasm contained mitochondria, the double membrane sheets of the rough and smooth endoplasmic reticula; in single cells, the Golgi apparatus was identified. Characteristic feature of neutrophils are specific intra-cellular granules that fill the cytoplasm. In the graying’s mesonephros, these granules had thin electron-dense stripes, fibrils, located along the granules. Two types of granules were found. The first type had fibrils located evenly along the granules. The second type had a lighter central part and dark fibrillar edges (Table 2, Fig. 2f).

Heterochromatin was both located along the nuclear membrane and diffused in the nucleoplasm. The nucleus was rounded, with a large amount of segmented heterochromatin. The cytoplasm was electron-light with a large amount of mitochondria, reticulum sheets; sporadically vesicles were noted. The visceral layer was made of podocytes, 4.18–7.89 μm in size. Podocytes were located on the outer side of the glomerular capillaries. The nuclei of the podocytes had large invaginations, a well-developed rough endoplasmic reticulum, and from three to five mitochondria. The thickness of the glomerular basement membrane was 0.18 ± 0.01 μm (Fig. 3a, 3b).

Ultrastructure analysis of the proximal tubules in the nephrons of graying showed two types of epithelial cells. The type I epithelial cells were located at the beginning of the proximal tubule; these were the largest cells in the proximal tubule. These epithelial cells had an elongated pyramidal shape, were tightly adjacent to each other, and had round-shaped nuclei located in the basal part of the cells. The cytoplasm was acinous and contained a large amount of mitochondria (Table 4, Fig. 3c). Basal cell membrane formed projections turning into the sheets of the smooth endoplasmic reticulum (Fig. 3f). These membrane projections were characteristic for all types of epithelial cells in all divisions of the tubule. Lysosomes and the large electron-dense secretory granules characteristic of this nephron segment were present (Table 4, Fig. 3e). In the apical part of the cells, adjacent to the brush border, there was a well-developed zone of endocytosis (Table 4, Fig. 3d). This zone was characterized by the presence of the tubulo-vesicular network (Fig. 3j). The epithelial cells of this type had the longest microvilli. Furthermore, single cells, the brush border of which was formed by cilia, were found there (Table 4, Fig. 3g). Epithelial cells having cilia had a slightly different ultrastructure. The cytoplasm of these cells was less acinous; the apical edge of the cells was more electron-dense than that of epithelial cells having microvilli (Fig. 3j).

Bowman’s capsule has the parietal layer and visceral layer and the Bowman’s space in between, into which the filtrate enters after passing through the filtration slits. In graying, the Bowman’s space was 3.81 ± 0.78 μm in size. The parietal layer was composed by a single layer of flat epithelial cells in contact with the basement membrane. Epithelial cells were elongated, with a centrally located nucleus. Heterochromatin was both located along the nuclear membrane and diffused in the nucleus. The cytoplasm contained mitochondria, reticulum sheets; sporadically vesicles were noted. The visceral layer was made of podocytes, 4.18–7.89 μm in size. Podocytes were located on the outer side of the glomerular capillaries. The nuclei of the podocytes had large invaginations, a well-developed rough endoplasmic reticulum, and from three to five mitochondria. The thickness of the glomerular basement membrane was 0.18 ± 0.01 μm (Fig. 3a, 3b).

Table 2
Dimensions (μm) and number of leucocyte subcellular structures in mesonephros of graying, Thymallus thymallus (x ± SE, n = 20)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lymphocytes</th>
<th>Plasma cells</th>
<th>Macrophages</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>6.17 ± 0.36</td>
<td>5.09 ± 0.41</td>
<td>11.07 ± 1.04</td>
<td>7.47 ± 0.93</td>
<td>15.35 ± 1.28</td>
</tr>
<tr>
<td>Number of mitochondria on a cell section</td>
<td>5.10 ± 0.23</td>
<td>4.36 ± 0.33</td>
<td>5.54 ± 1.34</td>
<td>4.49 ± 0.63</td>
<td>4.72 ± 0.40</td>
</tr>
<tr>
<td>Number of granules on a cell section</td>
<td>0.53 ± 0.11</td>
<td>0.31 ± 0.05</td>
<td>0.42 ± 0.06</td>
<td>0.20 ± 0.02</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 3
Dimensions (μm) and number of cells with a radial vesicle array, chloride cells, and their subcellular structures in the mesonephros of graying, Thymallus thymallus (x ± SE, n = 20)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cells with a radial vesicle array</th>
<th>Chloride cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>5.27 ± 0.67</td>
<td>4.78 ± 0.68</td>
</tr>
<tr>
<td>Nucleus size</td>
<td>4.02 ± 0.40</td>
<td>3.34 ± 0.15</td>
</tr>
<tr>
<td>Vesicle size</td>
<td>0.69 ± 0.08</td>
<td>0.48 ± 0.04</td>
</tr>
<tr>
<td>Number of vesicles on a cell section</td>
<td>4.50 ± 0.75</td>
<td>–</td>
</tr>
<tr>
<td>Mitochondrion size on a cell section</td>
<td>–</td>
<td>0.67 ± 0.09</td>
</tr>
<tr>
<td>Number of mitochondria on a cell section</td>
<td>–</td>
<td>6.33 ± 1.78</td>
</tr>
</tbody>
</table>

Bowman’s capsule has the parietal layer and visceral layer and the Bowman’s space in between, into which the filtrate enters after passing through the filtration slits. In graying, the Bowman’s space was 3.81 ± 0.78 μm in size. The parietal layer was composed by a single layer of flat epithelial cells in contact with the basement membrane. Epithelial cells were elongated, with a centrally located nucleus. Heterochromatin was located along the nuclear membrane and diffused in the nucleus. The cytoplasm contained mitochondria, reticulum sheets; sporadically vesicles were noted. The visceral layer was made of podocytes, 4.18–7.89 μm in size. Podocytes were located on the outer side of the glomerular capillaries. The nuclei of the podocytes had large invaginations, a well-developed rough endoplasmic reticulum, and from three to five mitochondria. The thickness of the glomerular basement membrane was 0.18 ± 0.01 μm (Fig. 3a, 3b).
A characteristic feature of the type II epithelial cells was the absence of secretory granules in the cytoplasm. Although the tubulo-vascular network was present, the endocytosis zone was smaller in comparison with the type I epithelial cells (Table 4, Fig. 3h, 3i). The brush border was shorter; it was formed by both cilia, from ciliated epithelial cells, and microvilli (Table 4). In the ciliated type II epithelial cells, the cytoplasm had a greater amount of larger mitochondria in comparison with the main type II epithelial cells.

The epithelial cells in the distal tubule were high and very wide in the base (Table 4). The nuclei of most cells occupied the central part, sometimes were shifted to the basal part. Heterochromatin was both concentrated at the periphery of the nucleus between nuclear pores and diffused over the entire surface. In the cytoplasm of these cells, a large number of membrane projections were present. Large elongated electron-dense mitochondria, located strictly along the longitudinal axis throughout the cell, were observed (Fig. 3j). By the number of mitochondria, the epithelial cells of the distal tubule were superior to the type I epithelial cells and inferior to the type II epithelial cells of proximal tubules. The endocytosis zone was absent. In the apical part of the cells, the blade-shaped cytoplasmic processes, facing the lumen of the tubule, were observed. Some processes were rugose (Fig. 3k).

**Discussion**

Physiological endpoints of interest, i.e. antioxidant enzyme activities and monooxygenase activities, are well established biomarkers of chemical exposure (Whyte et al., 2000; Sayeed et al., 2003; Atli & Carli, 2010; Sinha et al., 2014). Our results provide baseline data on superoxide dismutase, catalase, glutathione S-transferase, and ethoxyresorufin-O-deethylase activities in the grayling kidney. These endpoints are considered components of an “early-warning system” for fish health and thus can be used in further studies addressing the health status of the grayling population in the Urya River. In an attempt to conduct a discussion of the results from a comparative-evolutionary perspective, we used data obtained earlier for the grayling’s close freshwater relative, the Northern pike *Esox lucius* (Lapirova et al., 2017). Northern pike is a member of the Esociformes, the sister order to the Salmoniformes within the Protacanthopterygii superorder, and it takes an ancestral position to the Grayling and the Grayling-like species habitats. It is well established that ciliated segments of nephron (neck segment) lack the neck segment and a small amount of cilia, which were observed not only in the granulocyte differential, but also in the quantity of granules on the sections of neutrophils and eosinophils as well as in the structure of specific granules in neutrophils. By the quantity of granules on the sections of neutrophils and eosinophils, the grayling had a resemblance to the Northern pike rather than to the more closely related rainbow trout and vendace (Flerova & Balabanova, 2013). It was reported earlier (Flerova, 2017) that in the mesonephros of the Northern pike, the neutrophils had specific fibrillar granules, which could be conditionally divided into three types. In the mesonephros of grayling, the neutrophils had specific fibrillar granules with the ultrastructure similar to the type I and type II specific fibrillar granules of the Northern pike. The characteristic feature of the type III specific fibrillar granules was that the fibrils were located close to the central part of the cell. In neutrophils of rainbow trout and vendace, only one type of specific fibrillar granule was found, with a light central part and dark fibrillar edges (Flerova & Balabanova, 2013). It was presumed earlier that the differences in the quantity of fibrils and their distribution between the granules of neutrophils are associated with a stage of the granule maturation rather than the species-specificity (Flerova & Balabanova, 2013). The frequency of neutrophils having granules of a certain type on the sections of mesonephros and the quantity of granules on the sections of neutrophils and eosinophils are possibly characteristic to the cellular immunity functioning in the particular environmental conditions typical of the species habitats.

A lack of mesangial cells, small amount of podocytes on the sections of the renal corpuscle, and a thin basement membrane indicate the high glomerular filtration rate, which is characteristic of freshwater fishes (Ojeda et al., 2006). In grayling, the nephron does not possess a neck segment, which was observed earlier in the mesonephros of some fish (Ojeda et al., 2006; Flerova, 2017) and mammalian species (Ojeda & Icardo, 1991). On sections of the proximal tubules, ciliated cells were rare, and large amounts of the tubulo-vesicular network in the zone of endocytosis of the type II epithelial cells were observed. On sections of the distal tubules, short blade-shaped cytoplasmic processes, with large numbers of invaginations of cytoplasmic membrane, were witnessed. It is well established that ciliated segments of nephron (neck segment and ciliated cells of proximal tubules) are involved in the processing the fluid. The cilia move the urine toward the proximal tubule, reducing the pressure in the urinary space and, consequently, increasing the glomerular filtration rate (Ojeda et al., 2006). Development of the tubular-vesicular network promotes the reabsorption rate in the proximal tubules (Flerova, 2017). Lumen enlargement due to short blade-shaped cytoplasmic processes probably lowers the resistance to urinary flow. In the study of Na⁺/K⁺/2Cl⁻-cotransporter localization in the kidneys of an euryhaline salmonid, rainbow trout (Katoh et al., 2008), a lack of the neck segment and a small amount of cilia, which were mostly found in the tubule lumen, was recorded. Rainbow trout was lacking the Na⁺/K⁺/2Cl⁻-cotransporter, which mediates the electroneutral transport of Na⁺, K⁺, and Cl⁻ across epithelial membranes, in the basolateral membrane of the proximal tubules. In the distal tubules, the apically
located Na\(^+\)/K\(^+\)/2Cl\(^-\)-cotransporters were observed, which it has been suggested play a role in Na\(^+\), K\(^+\), and 2Cl\(^-\) absorption allowing the ions to be transported back into the body through basolateral-specific ion channels. Thus, the distal tubules could probably switch between secretion and absorption, depending on the osmoregulatory needs of the fish (Katoh et al., 2008). It is possible that the ultrastructural features observed in thegrayling mesonephros in our study are the cytological markers ofgrayling being adapted to euryhalinity since it can occur in brackish waters and on small river rifts in the tidal backwater zones.

Conclusion

Our study provides new data on morpho-functional characteristics of the mesonephros in the grayling. The degree of the hematopoietic tissues development was determined. New data on the ultrastructure of leukocytes, cells with a radial vesicle array, chloride cells, and nephron tissue development was determined. New data on the ultrastructure of the mesonephros in the grayling. The hematopoietic system of grayling being adapted to euryhalinity since it can occur in brackish waters and on small river rifts in the tidal backwater zones.

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