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Microanatomy and ultrastructure of kidney interstitial cells and nephron in brown trout (*Salmo trutta* Linnaeus, 1758) at different stages of the life cycle

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Abstract

The study focuses on the microanatomy and ultrastructural changes in the trunk kidney interstitium cells and nephrons in parr, smolt and spawning brown trout *Salmo trutta* Linnaeus, 1758 sampled in Luga River and Solka River, the tributaries of the Baltic Sea. Regardless of the type of cells or their structure, there were changes in their areas and the number and structure of organelles responsible for the transport, synthetic and energetic function of cells. Our data on the morphology of the nephron combined with data on its physiology suggest a fundamental change in kidney function during the parr-smolt transformation before migration; this could be a preadaptation for a successful life in saltwater where urine output is sharply reduced. Thus, detected structural features of the trunk kidney in brown trout *S. trutta* are cytological markers of the migration process. The numbers of lymphocytes, neutrophils and eosinophils with segmented nuclei increased from parr to smolts and then to spawners; only monotypic specific granules in neutrophils were found in smolts and spawners. Cells with radially arranged vesicles were described for the first time in brown trout *S. trutta* renal interstitium. Their origin has not yet been established. The shape of these cells changed from spherical to trihedral during fish maturation. All the above ultrastructural changes of renal interstitium cells could be considered cytological markers of cell maturity.

Keywords: fish, brown trout *Salmo trutta*, migration, microanatomy, ultrastructure, mesonephros

Introduction

Anadromous salmonids have large distribution areas, divided into “spawning” (riverine) and “feeding” (marine) habitats. Within the riverine habitat, salmonids spawn, grow and develop, preparing for the seaward migration. Marine habitat offers extensive feeding opportunities, providing salmonids with energy for somatic growth, gonad development and return to natal rivers. Anadromous salmonids experience a complex of cyclic factors cueing changes in related

physiologic conditions. Such changes begin during the offshore migration and end during the spawning migration to the natal river. Anadromous salmonids exhibit wide environmental tolerance during their ontogenetic habitat shifts (Folmar and Dickhoff 1980; Tanguy et al. 1994; Rønneseth et al. 2005; Kantserova et al. 2017). The overwhelming majority of studies on salmonids migration are limited to physiological changes of the hyperosmoregulatory system, and only a few studies cover the ultrastructural changes in the nephron (Vinnichenko et al. 1975; Folmar and Dickhoff 1980; Maksimovich et al. 2000). In the comparative-evolutional aspect, it is essential to study the structural-functional features of the kidney, which not only plays a key role in osmoregulation but significantly contributes to the non-specific protection system. Furthermore, the kidney is a unique organ of hematopoiesis, where the cells undergo proliferation and maturation, and completion of all the stages of the immune response regardless of other lymphoid organs are possible (Becker et al. 2001; Zapata et al. 2006; Resende et al. 2010). There are surveys related to the adaptive changes in the nephron tubules occurring in juvenile salmonids of the Pacific Ocean during migration to the sea and salmonids during spawning migrations (Maksimovich et al. 2000; Maksimovich 2008). The characteristic features of the essential kidney excretory components in adult individuals of *Salmo trutta* f. *fario*, depending on sex and season, were revealed at a light-microscopic level using stereological methods (Resende et al. 2010). To the best of our knowledge, there are no comprehensive studies on the structural changes in salmonids' mesonephros caused by the adaptation to the environment.

The use of brown trout *S. trutta* Linnaeus 1758 as a model object has become more common with the advent of fish farming, lake and stream stocking and pollution monitoring due to declining natural populations of brown trout *S. trutta* (Anderson and Loewen 1975; Tanguy et al. 1994; Freyhof 2011; Helcom Red List *Salmo trutta* 2013; Resende et al. 2010; ICES 2017). Based on the IUCN criteria, HELCOM (Baltic Marine Environment Protection Commission – Helsinki Commission) categorises brown trout *S. trutta* as a vulnerable (VU) species (Helcom Red List *Salmo trutta* 2013). In Russia, anadromous, lacustrine and riverine lineages of the Baltic Sea brown trout *S. trutta* are considered endangered (EN) (Red Data Book of the Russian Federation (animals) 2001).

This study focuses on the microanatomy and ultrastructural changes of trunk kidney cells in parr, smolt and adult (spawning) anadromous Baltic Sea brown trout *S. trutta*. Achieving this goal is significant both theoretically and practically since these data expand the knowledge on the structure of mesonephros of lower vertebrates and could be used in environmental monitoring studies.

75 **Materials and Methods**

76 Fish and sampling

77 This study was performed in compliance with the local laws (permission No. 78 2017 03 3049 dated
78 11.04.2017); the brown trout *S. trutta* were collected within the framework of the studies of Berg State Research
79 Institute on Lake and River Fisheries. Fish were sampled from the Luga River and its tributary, the Solka River (Figure
80 1), which flow into the Gulf of Finland, the easternmost stretch of the Baltic Sea. The Luga River flows for 346 km; the
81 total drainage basin is 13,200 km². In terms of the chemical composition, river waters belong to the bicarbonate class;
82 total dissolved solids concentrations vary from 120-200 mg/l during high water in spring to 300-400 mg/l during
83 baseflow.

84 Parr and spawners were collected from the Solka River sampling site using gill nets on October 25, 2017. Smolts
85 were caught using a floating trap net at the Luga River sampling site on May 19, 2017. Fish were treated according to
86 the procedure for the use of fishes in research of the Laboratory of Physiology and Toxicology of Aquatic Animals,
87 Papanin Institute for Biology of Inland Waters Russian Academy of Sciences, consistent with the CCAC Guidelines on
88 the Care and Use of Fish in Research, Teaching and Testing (Canadian Council on Animal Care, 2005) and the Guide
89 for the Care and Use of Laboratory Animals (National Academy of Sciences, 2011). Each individual, one after the
90 other, was immobilised by a stunning blow to the head, quickly measured in fork length (FL) and subjected to cervical
91 transection by an experienced person. After that, an individual was dissected and eviscerated; sex was determined
92 visually. Samples were as follows: parr – three males (FL 12-19 cm) and two females (FL 18, 20.5 cm); smolts – four
93 females (FL 12-16 cm); spawners – one male (FL 31 cm) and two females (FL 34, 55 cm).

94 Five portions of the medial part of the trunk kidney were collected from each fish. Samples were fixed in 2.5%
95 glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide for 1 hr, dehydrated in graded acetone and
96 propylene oxide and embedded in Araldite (Electron Microscopy Sciences, USA) (Timakova et al. 2014).

97 Trunk kidney microanatomy

98 Semithin sections (2-3 µm) were cut with a UMTP-3 microtome (Russia). Ten sections from each of the five
99 portions of the kidney were prepared. Sections were stained with methylene blue. A total of 600 microscopic sections
100 were made; a digital image was obtained for each one. Digital images were taken using a Micromed-6 light microscope
101 (Russia) and processed using Image Tool 3.0 software. The outer diameters of the blood vessels, renal corpuscles and
102 tubules were measured. The area of renal interstitium was calculated as the difference between the total area of the

section and the sum of the areas of blood vessels, renal corpuscles and tubules and reported as a per cent value (Timakova et al. 2014). From each individual, 20 measurements were obtained for each measured structure.

Trunk kidney ultrastructure

Ultrathin sections were cut using a Leica EM UC7 Ultracut (Germany). Five sections from each of five portions of the kidney were prepared. A total of 300 sections were made. Sections were stained with uranyl acetate and lead citrate and analysed using a JEM 1011 transmission electron microscope (Japan). A digital image was obtained for each section. Measurements of areas of cells, organelles and inclusions, lengths of the endocytosis zone and brush border, cilium and microvillus diameters were performed on digital images using Image Tool 3.0 software; from each individual, 10 measurements were obtained for each measured structure. On digital images of cell sections, the number of observed mitochondria, specific granules, vesicles and secretory granules were determined.

Statistical analysis

To determine the differences between fish groups, statistical analysis was performed in two steps. First, replicates of the data were obtained for each individual; mean values and standard error of the mean were calculated. No statistically significant differences were found between male and female parr and spawners; therefore, males and females were analysed together at the next step. At this point, mean values and standard error of the mean were calculated for a set of individuals of one ontogenetic stage (parr, smolts, or spawners). Statgraphics Plus software was used to analyse data sets. Non-parametric tests were applied, namely, Kruskal–Wallis ANOVA with post hoc Wilcoxon–Mann–Whitney test. The significance level was set to 0.05 ($p < 0.05$).

Results

The trunk kidney of parr, smolt and spawning brown trout *S. trutta* is composed of the renal interstitium, formed by hematopoietic tissue surrounding nephrons and small blood vessels; renal interstitium also forms small islands located closer to the lateral side of the organ. The proportion of renal interstitium in the total area of kidney tissue was 44–51% (Table 1). The comparison of three ontogenetic stages of brown trout *S. trutta* has shown that the values of the outer diameters of renal corpuscles in parr and spawners are greater than those of smolts. The renal corpuscle is followed by a tubule divided into proximal and distal sections. The average diameters of the proximal tubules are similar. The diameter of distal tubules in parr is larger than in smolts and spawners. The outer diameters of blood vessels of parr, smolts and spawners are similar (Table 1, Figure 2).

At the ultrastructural level, in the renal interstitium, leukocytes of various stages of maturity and cells with radially-arranged vesicles were found in brown trout *S. trutta* of all studied ontogenetic stages. The renal interstitium of smolts and spawners also contains chloride cells.

Lymphocytes are spherical cells that have the smallest size among leukocytes (cells area 18-32 μm^2). No statistically significant differences were found between the lymphocyte cell areas of parr, smolts and spawners (Table 2). The characteristic feature of lymphocytes is a large nucleus occupying almost the whole cell. Most lymphocytes in parr have spherical nuclei. In smolts, lymphocyte nuclei are spherical or lobed. In spawners, the majority of lymphocytes have bi-lobed nuclei. Heterochromatin is evenly distributed throughout the nuclei, regardless of their shape. No statistically significant differences were found between the area of the nuclei. The fine rim of cytoplasm is filled with numerous loose ribosomes, separate stacks of rough endoplasmic reticulum and mitochondria (Figures 3a, b, c). In smolts, the average number of mitochondria significantly exceeds those found in parr and spawners. In smolts, mitochondria are larger than in parr and spawners (Table 2).

As a rule, plasma cells are oval with an area of 45-91 μm^2 and an eccentrically shifted nucleus (14-42 μm^2), spherical or lobed, regardless of the ontogenetic stage. The areas of cells and nuclei of smolts significantly exceed those of spawners (Table 2). The major part of heterochromatin adjoins the inner membrane of the nuclear envelope. Apart from it, separate clods are evenly located across the sections of the nucleus. The cytoplasm contains stacks of well-developed rough endoplasmic reticulum ($0.13 \pm 0.06 \mu\text{m}$ wide in parr, $0.19 \pm 0.03 \mu\text{m}$ – in spawners and $0.46 \pm 0.10 \mu\text{m}$ – in smolts), loose ribosomes, electron-dense lysosomes and mitochondria (Figures 3d, e, f). The average number of mitochondria in plasma cells of brown trout *S. trutta* at different stages of ontogenesis varied from 3.50 ± 0.65 in cells of spawners to 7.33 ± 0.67 in cells of smolts. The area of mitochondria was the largest in smolt cells in comparison with parr and spawners (Table 2).

Macrophages are the largest spherical, bean-shaped or band-shaped leukocytes (Table 2), with eccentrically located nuclei, regardless of the ontogenetic stage of trout. The major part of heterochromatin is located on the periphery adjoining the inner membrane of the nuclear envelope. Heterochromatin forms clods or threads on sections of the central part of the nucleus. The cytoplasm contains phagosomes, which occupy almost the entire cell volume. The phagosomes of smolts are significantly larger ($9.11 \pm 2.44 \mu\text{m}^2$), in comparison with phagosomes of parr ($6.25 \pm 2.19 \mu\text{m}^2$) and spawners ($3.06 \pm 1.29 \mu\text{m}^2$). The number of phagosomes per cell also varies considerably between the stages of ontogenesis. Smolts show the largest number (19.00 ± 3.00) compared to parr (5.33 ± 1.45) and spawners (9.00 ± 1.53). The cytoplasm of macrophages is also packed with mitochondria, short sheets of rough endoplasmic reticulum, loose ribosomes, lysosomes and small light vesicles. In smolts, mitochondria are larger than in parr and spawners (Table 2). In parr, the cytoplasm of macrophages contains electron-dense pigment granules (Figures 3g, h). In addition

to solitary macrophages, brown trout *S. trutta* renal interstitium contains macrophage aggregates, also known as melano-macrophage centres, which are the distinctive groupings of tightly packed macrophages enclosed in a common envelope with inclusions of electron-dense pigment granules (Figure 3i). The melano-macrophage centres are found in spawners ($307 \pm 38.6 \mu\text{m}^2$) and parr ($303 \pm 46.70 \mu\text{m}^2$) being larger than macrophage aggregates of smolts ($198 \pm 47.5 \mu\text{m}^2$).

Neutrophils are the most abundant granulocytes, or polymorphonuclear leukocytes, in the brown trout *S. trutta* renal interstitium. Neutrophils are spherical cells with eccentrically shifted nuclei at all three ontogenetic stages. The majority of neutrophils in the renal interstitium of parr are band neutrophils; some neutrophils have segmented nuclei with two to three sections. Most of the neutrophils in the renal interstitium of smolts are segmented ones; 80% have a nucleus with three sections. In the renal interstitium of spawners, band and segmented neutrophils are present in equal shares. Almost all segmented neutrophils have nuclei with two sections. Cloddy heterochromatin is located mainly on the periphery adjoining the inner membrane of the nuclear envelope; separate clods are found in the central part of the nucleus. Parr and spawners have larger nuclei compared to smolts (Table 2). Mitochondria have a high density in neutrophils at each ontogenetic stage. The average sizes of mitochondria in the neutrophil sections of parr and spawners are equal and less than that of smolts (Table 2). Neutrophils of spawners have better developed rough endoplasmic reticulum compared to immature fish. The cytoplasm of neutrophils in parr has specific granules of two types: the first, electron-dense with fibrils located along the granules, and the second, more electron-light in the central part and dark fibrillar edges. The cytoplasm of neutrophils in smolts and spawners contains only granules of the second type. The size and number of granules in neutrophils of smolts are larger than those found in parr and spawners (Table 2, Figures 4a, b, c).

Eosinophils, a type of granulocytes, contain spherical electron-dense granules of homogenous structure regardless of the trout's ontogenetic stage (Table 2, Figures 4d, f). The number of granules on the cell sections of smolts is over four times higher than those found in parr and spawners. The average size of eosinophils granules in the renal interstitium is also greater in smolts compared to parr and spawners (Table 2). Eosinophils, spherical cells, have eccentrically located nuclei at each studied ontogenetic stage. Most eosinophils in parr are immature cells with spherical nuclei; only 10% are band cells. Almost all eosinophils in the renal interstitium of smolts are band cells. In spawners, there is an equal amount of band and segmented eosinophils. All segmented cells have bi-lobed nuclei. Cloddy heterochromatin is mainly found on the periphery adjoining the inner membrane of the nuclear envelope; separate clods are found in the central part of the nucleus (Figures 4d, f). In eosinophils, mitochondria are characterised by a high density at each ontogenetic stage. The average number of mitochondria in smolts is greater than parr and spawners (Table 2). In spawners, there are some eosinophils with atypically structured granules (Figure 4e).

Cells with radially arranged vesicles are of spherical or trihedral shape. Almost all cells with radially arranged vesicles in the renal interstitium of parr are spherical with eccentrically located spherical nuclei (Figure 5a). Smolts and spawners possess trihedral cells with radially arranged vesicles. In smolts, cells with radially arranged vesicles have spherical or bean-shaped nuclei, whereas, in spawners, these cells are band cells (Figure 5c). In these cells, regardless of the ontogenetic stage of brown trout *S. trutta*, the heterochromatin is very condensed. The cytoplasm is packed with short stacks of the smooth endoplasmic reticulum and mitochondria. There are vesicles and one or two mitochondria next to them in the apical part of the cells (Figure 5b). Vesicles in cells of smolts are larger in comparison with those of parr and spawners (Table 3).

Chloride cells were identified only in the renal interstitium of smolts and spawners. They are long and narrow cells (Table 3), localised both diffusely in the parenchyma of the kidney or surround the blood vessels and tubules of the nephrons. The spherical nucleus is located closer to the periphery of the cell; heterochromatin is slightly condensed. The cytoplasm is electron-light with a large number of mitochondria. In smolts, mitochondria number is more than twice higher than the number of those in spawners (Table 3). There are electron-dense granules and two double-membrane organelles, tubular and rough endoplasmic reticulum, in both smolts and spawners. Single tubules are distributed throughout the cell; stacks of the rough endoplasmic reticulum are mainly located in the perinuclear area and are few (Figures 5d, e, f).

The nephron consists of a renal corpuscle and renal tubules. The renal corpuscle in brown trout *S. trutta* at each studied ontogenetic stage is similarly structured and consists of a group of capillaries and a Bowman's capsule. The Bowman's capsule has two layers, parietal and visceral, forming urinary space, which is $5.31 \pm 0.50 \mu\text{m}$ in parr, $4.17 \pm 0.35 \mu\text{m}$ in smolts and $5.31 \pm 0.68 \mu\text{m}$ in spawners. The parietal layer is formed by a basement membrane lined by squamous epithelial cells with centrally located nuclei. The visceral layer consists of podocytes located on the basement membrane $0.66 \pm 0.11 \mu\text{m}$ thick in parr, $1.40 \pm 0.30 \mu\text{m}$ in smolts and $0.41 \pm 0.08 \mu\text{m}$ in spawners. The basement membrane is common for podocytes and capillaries (Figures 6a, b).

Proximal tubules of the nephron have epithelial cells typical for this unit of the nephron. They are distinguished from the cells of the distal tubule by the presence of a brush border. Ultrastructure analysis of the proximal tubule shows two types of epithelial cells. Type I epithelial cells are located at the beginning of the proximal tubule; these are the largest cells in the proximal tubule. These epithelial cells are characterised by an elongated pyramidal shape and tightly stacked smooth endoplasmic reticulum. In smolts, cell area and height are smaller compared to parr and spawners (Table 4). The average height of cells is $14.04 \pm 0.86 \mu\text{m}$ in smolts, $17.9 \pm 1.23 \mu\text{m}$ in parr and $19.3 \pm 0.79 \mu\text{m}$ in spawners. These epithelial cells have spherical nuclei located in the basal part of the cells. In addition, the basal part of the cell contains numerous curls of the smooth endoplasmic reticulum. These infoldings are better developed in

smolts (Figures 6e, g, f). The cytoplasm is packed with mitochondria, the size and number of which are greater in smolts. There are lysosomes and the large electron-dense secretory granules characteristic of this nephron segment. The largest secretory granules were found in cells of smolts (Table 4). There is a well-developed zone of endocytosis in the apical part of the cells adjacent to the brush border. This zone has a tubulovesicular network. The length of the endocytosis zone does not depend on the ontogenetic stage of brown trout *S. trutta* (Table 4). The brush border is formed by numerous microvilli facing the tubule lumen. The brush border length and microvilli diameter do not depend on the ontogenetic stage of the brown trout *S. trutta* (Table 4, Figures 6c, d, e). Among type I epithelial cells at each ontogenetic stage of brown trout *S. trutta*, there are single cells with a brush border formed by cilia of the same diameter. In smolts, such ciliated epithelial cells are rare compared to parr and spawners. The ultrastructure of type I ciliated epithelial cells has several differences. These cells possess lighter cytoplasm; the endocytosis zone is poorly developed; there are only single tubules; the number of vesicles is small; mitochondria are concentrated in the apical part of the cells below the ciliary rootlets (Figures 4h, i).

In general, type II epithelial cells are similar to type I epithelial cells. The area and height of type II epithelial cells are significantly smaller in smolts compared to parr and spawners (Table 4). The height of these cells is $11.89 \pm 1.07 \mu\text{m}$ in smolts, $17.26 \pm 1.95 \mu\text{m}$ in parr and $14.05 \pm 0.77 \mu\text{m}$ in spawners. Type II epithelial cells also have spherical nuclei located in the basal part of the cells. The nuclei sizes are similar among the ontogenetic stages of brown trout *S. trutta*. In parr and smolts, heterochromatin in type II epithelial cells is less condensed than in type I epithelial cells. In spawners, heterochromatin is electron-dense and located mainly on the periphery adjoining the inner membrane of the nuclear envelope; separate heterochromatin clods are evenly located across the nucleus sections. The cytoplasm is filled with many mitochondria, which are located along the longitudinal axis of the cells. In the basal part of the cell, these mitochondria are elongated. In the apical part of cells, mitochondria are less ordered and have a spherical shape. In smolts, mitochondria of both the basal and apical parts of the cell are larger than in parr and spawners. In smolts, the average number of mitochondria in a cell is more than three times higher than the number of mitochondria in parr and spawners (Table 4). A characteristic feature of type II epithelial cells is the absence of secretory granules in the cytoplasm. The endocytosis zone and brush border lengths are similar at different ontogenetic stages of brown trout *S. trutta* (Table 4). However, the diameters of microvilli in spawners and smolts are much larger than in parr (Table 4). There are single cells of ciliated epithelium among the type II epithelial cells in brown trout *S. trutta* at each stage of ontogenesis. In smolts, such ciliated epithelial cells are rare in comparison with parr and spawners (Figures 7a, c, e).

Distal tubules of the nephron are lined by the tallest and wide epithelial cells among tubule cells. The height of cells is $20.24 \pm 0.45 \mu\text{m}$ in parr, $18.46 \pm 0.65 \mu\text{m}$ in smolts and $20.93 \pm 0.86 \mu\text{m}$ in spawners. The area and height of cells are similar (Table 5). Spherical nuclei are shifted to the basal part of the cells (Figures 7b, g). Heterochromatin in

these cells is very condensed; the cytoplasm is filled with loose ribosomes and vesicles. Smooth endoplasmic reticulum forms infoldings, which are better developed in smolts (Figures 7d, f, h). There are large elongated electron-dense mitochondria located along the longitudinal axis of the cells. In the apical part of the cells, mitochondria are spherical and less ordered (Table 5). In smolts, the area of mitochondria in the apical part of the cell is larger in comparison with parr and spawners; the average number of mitochondria in the cells is more than twice the number of mitochondria registered in parr and spawners (Table 5). There is no endocytosis zone. In the apical part of the cells, lobed cytoplasmic processes are located facing the lumen of the tubule. In smolts, these cytoplasmic processes contain many vesicles and several mitochondria (Figures 7b, g, h).

Discussion

Changes in the renal activity of salmonids during their migration are directly related to general processes of rearrangement of all functional systems in the organism. At the early stages of individual development in freshwater, salmonids can change the character of osmoregulation in order to migrate to the sea, whereas sexually mature fish during their spawning migration return to natal rivers and replace the hypoosmotic regulation by the hyperosmotic one. Various organs and systems are involved in osmoregulation: kidneys, gills, digestive tract, urinary bladder and endocrine glands (Folmar and Dickhoff 1980). Results obtained in our study illustrate the changes in trunk kidney microanatomy and ultrastructure in parr, smolt and spawning brown trout *S. trutta* related to seaward and spawning migrations, as well as fish ageing.

Both the structure of the nephron and the ultrastructure of the renal corpuscle and renal tubules of the parr and spawners of brown trout *S. trutta* correspond to the results obtained for freshwater trout (Anderson and Loewen 1975). Smolts have smaller outer diameters of renal corpuscles and tubules, reduced urinary space, thicker basement membrane of the Bowman's capsule. Earlier, it was shown for *Etroplus maculatus* (Bloch, 1795) and *Alburnus escherichii* Steindachner, 1897 that adaptation to a hyperosmotic environment leads to a decrease in the diameter of Bowman's capsules, accompanied by a decrease in the glomerular filtration rate, which indirectly indicates a decrease in the formation of primary urine. (Virabhadrachari 1961; Oğuz 2015). The smaller area and height of epithelial cells of the proximal tubules of smolts indicate a low glomerular filtration rate typical for marine fish (Vinnichenko 1975; Natochin 1976; Talbot 1989; Maksimovich et al. 2000). It is known that the urine output of euryhaline teleosts is a resultant of adjustments in two basic renal mechanisms: glomerular filtration and tubular reabsorption. In freshwater, glomerular filtration is high and tubular reabsorption is low, while in seawater, the filtration rate is significantly reduced, and the reabsorption is accelerated. The anadromous salmon in fresh water and saltwater behave like other euryhaline fishes, but the two fundamental mechanisms have not been carefully assessed during the parr-smolt

transformation. One of the few relevant studies indicated an almost 50% reduction in the urine flow in *Oncorhynchus mykiss* (Walbaum 1792) smolts in comparison with pre- and post-smolts. The glomerular filtration rate was about 50% lower in smolts than in pre- and post-smolts (Hoar 1988). Unfortunately, there are no data on the speed of smolts' adaptation to saltwater. However, there was a study conducted by Talbot et al. (1992) on urine regulation in Atlantic salmon *Salmo salar* Linnaeus, 1758 returning to the sea after spawning in freshwater. Such fish, known as kelts, had a limited ability to hypoosmoregulation. This study showed that freshwater-adapted kelts demonstrate rapid adaptation (within 48 h) in osmoregulatory parameters to values characteristic of seawater-adapted salmonids. The urine flow rate fell from 1.2 to 0.2 mL×kg⁻¹×h⁻¹ within 24 h. Over the same period, urine osmolality increased from 48 mosmol×kg⁻¹ to become isosmotic with the plasma, and Mg²⁺ secretion by the kidney tubules elevated the urine concentration from 0.5 to 100 mmol×L⁻¹ (Talbot et al. 1992). Our study has shown that ultrastructural changes in the nephron, which affect the glomerular filtration rate and the amount of urine produced in smolts, occur even in freshwater. Our data on the morphology of the kidney combined with the physiological principles suggest a fundamental change in kidney function during the parr-smolt transformation before migration; this could be preadaptive for a successful life in saltwater, where urine output is sharply reduced.

There is evidence that in the spawning coho salmon *Oncorhynchus kisutch* (Walbaum, 1792), urine flow exhibited a tenfold increase from a mean of 0.406 mL/(kg×h) in saltwater to a mean of 4.65 mL/(kg×h) in freshwater. The glomerular titration rate increased from a mean of 1.48 mL/(kg×h) in saltwater to a mean of 9.06 mL/(kg×h) in freshwater (Miles 1971). These data are consistent with the results of our study, which showed that the morphology of mesonephros of parr and spawners caught in the spawning grounds was similar.

Ultrastructural changes in epithelial cells of proximal and distal tubules in smolts, compared to parr and spawners, such as increased size and number of mitochondria and smooth endoplasmic reticulum enlargement, might be a result of increased secretion of bivalent ions (Mg²⁺, Ca²⁺, SO₄²⁻), phosphates and reabsorption of water and sodium. Ions from the interstitial fluid enter the cell through the basement membrane, move towards the apical membrane and through it and enter the lumen of the tubule. The smooth endoplasmic reticulum is directly related to this mechanism since this cell structure provides the molecular basis for the operation of ion pumps (Natochin 1976). The distal tubules of rainbow trout, a euryhaline salmonid species, contain apically located Na⁺/K⁺/2Cl⁻ cotransporters, which were suggested to 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 (Kato et al. 2008). Probably, due to this mechanism, a large number of vesicles along with mitochondria occurred in the lobed cytoplasmic processes of epithelial cells in the distal tubules of the nephron in smolts of brown trout *S. trutta*.

Earlier studies on humpback salmon *Oncorhynchus gorbuscha* (Walbaum, 1792) showed that after it migrated from sea to the river, the blood plasma osmolality remained almost unchanged for a relatively long time, up to spawning, which allowed the renal cells to function with lower metabolic rate (Natochin 1976; Beyenbach 1995). This may result in a decrease in the smooth endoplasmic reticulum, size and number of mitochondria in epithelial cells of the proximal and distal tubules up to their complete absence in the lobed cytoplasmic processes of epithelial cells of the distal tubules of spawning brown trout *S. trutta*.

Another cellular mechanism of ion regulation involves chloride cells. Since the initial description of the chloride cell, there have been many histochemical and biochemical studies, which suggest that these mitochondria-rich cells are very likely engaged in the active transport of ions (Folmar and Dickhoff 1980). The cytoplasm of the ion-transporting cells in marine fish contains more mitochondria than freshwater ones since less energy is expended on the absorption of monovalent ions in freshwater fish than on the salts excretion in marine fish (Uchida et al. 1996; Flerova and Chuyko 2021). This might be true for the trunk kidney of brown trout *S. trutta* since the chloride cells in smolts are characterised by a larger number of mitochondria compared to spawners. Since there were no chloride cells in the renal interstitium of parr, these cells apparently form later in the ontogeny of the trout.

During smoltification, the kidney, gill, intestine and other osmoregulatory tissues, will pre-adapt for life in seawater, while the fish is still in freshwater (Hoar 1988; Talbot et al. 1992; Martemyanov 2016). Thus, all structural features of the trunk kidney in smolts are cytological markers of the migration process. During the smoltification, these changes occur in fish prior to reaching seawater.

The leukocyte ultrastructure analysis showed that parr, smolts and spawners mainly differ in mitochondria sizes in cells of all types except eosinophils, development of the rough endoplasmic reticula in plasma cells, number and sizes of phagosomes in macrophages, as well as number and sizes of granules in neutrophils and eosinophils. The size of mitochondria registered in the agranulocytes of smolts of brown trout *S. trutta* is more typical for marine fish (Flerova 2016). Therefore, it can be assumed that an increase in the size of leukocytes mitochondria during the transformation from parr to smolt, together with an increase in the area of the rough endoplasmic reticulum in plasma cells, indicates the activation of cellular immune systems under increased osmotic load. Furthermore, a significant decrease in the area and an increase in the number of specific granules in neutrophils and eosinophils and an increase in the size and number of phagosomes in macrophages also indirectly confirms the activation of the cell-mediated immunity. Whereas, in brown trout *S. trutta* returning to the natal river to spawn, the development of rough endoplasmic reticulum in neutrophils and the occurrence of eosinophils with an atypical granule structure indicate the non-specific immunity activation (Balabanova and Zabotkina 1988).

In the renal interstitium of smolt and spawning brown trout *S. trutta*, cells with radially arranged vesicles were found. These cells are rarely found, not in all fish species and representatives of the same species. Among closely related species, cells with radially arranged vesicles were described in the atlantic salmon *S. salar*, rainbow trout *O. mykiss* and grayling *Thymallus thymallus* (Linnaeus, 1758). Their origin has not yet been established; it is assumed that this type of cells is involved in the defence response since they are localised in the hematopoietic organs, peripheral blood, perinatal fluid and intestine of fish (Ferguson 1976; Balabanova 2006; Flerova et al. 2019).

The numbers of lymphocytes, neutrophils, eosinophils and cells with radially arranged vesicles having spherical nuclei decrease from parr to smolts and then to spawners, while the numbers of those cells with segmented nuclei increase (intermediates are observed with lobed, bean-shaped and horseshoe-shaped nuclei), and cells with radially arranged vesicles change from spherical to trihedral. Only monotypic specific granules occur in neutrophils of smolts and spawners. These changes are apparently associated with the age-related changes in brown trout *S. trutta* rather than the physiological processes of anadromous migration. All the above ultrastructural changes are considered the cytological markers of cell maturity (Ferguson 1976; Ellis 1977; Balabanova 2006; Flerova and Balabanova 2013; Andreyeva et al. 2017).

Physiological mechanisms of ionic and osmotic regulation are activated with a change in the habitat of brown trout *S. trutta* performing anadromous migrations, which is provided by the high adaptive capacity of the cellular structures of the mesonephros. Cytological rearrangements occur both in the nephrons and in the cells of the interstitium. Regardless of the cell types or structure, there are changes in cell areas and the number and structure of organelles responsible for the transport, synthetic and energetic function of cells.

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Competing interests

The authors declare there are no competing interests.

Contributors

E.F. - Conceptualisation, Project administration, Investigation, Funding acquisition, Writing – original draft, Writing – review & editing. V.Y. – Investigation, Writing – review & editing. Y.S. - Formal Analysis, Writing – review &

editing. D.S. – Resources, Methodology, S.T. – Resources, Methodology, Funding acquisition. A.M. – Investigation, Writing – review & editing.

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Data availability

Raw data generated during the current study are available from the corresponding author on reasonable request.

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Figure captions

Fig. 1. Location of sampling sites on the Luga River (1) and the Solka River (2). Figure was created using SAS.Planet version 200606 (<http://www.sasgis.org>) and assembled from the following data sources: base map (AI-

Hamdani et al. 2007); contour lines from NOAA National Geophysical Data Center ETOPO1 (Amante and Eakins 2009); shoreline (Harff et al. 2011).

Fig. 2. Microanatomy of the trunk kidney in parr (*a*, *b*), smolt (*c*) and spawning (*d*) brown trout *S. trutta*. Main structural units: blood vessel (1), renal interstitium (2), renal corpuscle (3), proximal tubule (4), distal tubule (5).

Fig. 3. Ultrastructure of agranulocytes (mononuclear leukocytes) in the renal interstitium of the brown trout *S. trutta*. Lymphocyte with a spherical nucleus in a parr (*a*); lymphocyte with a lobed nucleus in a smolt (*b*), lymphocyte with a segmented nucleus in a spawner (*c*); plasma cell in a parr (*d*); plasma cell in a smolt (*e*); plasma cell in a spawner (*f*); cytoplasm of a macrophage in a parr (*g*); macrophage in a spawner (*h*), melano-macrophage centre in a smolt (*i*). Units: nucleus (1), heterochromatin (2), mitochondria (3), rough endoplasmic reticulum (4), phagosomes (5), melanin granules (6), granulocyte aggregate (7).

Fig. 4. Ultrastructure of granulocytes (polymorphonuclear leukocytes) in the renal interstitium of the brown trout *S. trutta*. Neutrophil with a horseshoe-shaped nucleus in a parr (*a*); specific granules of neutrophil in a parr (*b*); neutrophil with a segmented nucleus in a spawner (*c*); eosinophil with a spherical nucleus in a parr (*d*); segmented eosinophil with atypical granules in a spawner (*e*); band eosinophil in a smolt (*f*). Cell units: nucleus (1), heterochromatin (2), mitochondria (3), rough endoplasmic reticulum (4), specific granules (5).

Fig. 5. Ultrastructure of cells with radially arranged vesicles and chloride cells in the renal interstitium of the brown trout *S. trutta*. Cell with radially arranged vesicles in a parr (*a*); cytoplasm of a cell with radially arranged vesicles in a smolt (*b*); cell with radially arranged vesicles in a spawner (*c*); cytoplasm of a chloride cell in a smolt (*d*); chloride cell in a smolt (*e*); cytoplasm of a chloride cell in a spawner (*f*). Cell units: nucleus (1), heterochromatin (2), mitochondria (3), radially arranged vesicles (4), cell centre (5), rough endoplasmic reticulum (6), tubular endoplasmic reticulum (7), electron-dense granules (8).

Fig. 6. Ultrastructure of the renal corpuscle and proximal tubule in the trunk kidney of brown trout *S. trutta*. Part of renal corpuscle in a parr (*a*); podocyte in a spawner (*b*); part of the proximal tubule in a parr (*c*); endocytosis zone and a portion of the brush border in a parr (*d*); basal part of type I epithelial cell of the proximal tubule in a parr (*e*); basal part of type I epithelial cell of the proximal tubule in a smolt (*f*); basal part of the proximal tubule with type I epithelial cells in a spawner (*g*); ciliated epithelial cell in a spawner (*h*); cilia (*i*). Cell units: urinary space (1), basement membrane (2), squamous epithelial cell (3), podocytes (4), nucleus (5), heterochromatin (6), endothelial cell (7), capillary (8), brush border (9), epithelial cell of proximal tubule (10), mitochondria (11), smooth endoplasmic reticulum (12), endocytosis zone (13), secretory granules (14), microvilli (15), cilia (16), tubular endoplasmic reticulum (17), vesicle (18).

Fig. 7. Ultrastructure of the proximal and distal tubule in the trunk kidney of brown trout *S. trutta*. Part of the proximal tubule with type II epithelial cells in a parr (*a*); part of the distal tubule in a parr (*b*); basal part of II type epithelial cell of the proximal tubule in a parr (*c*); basal part of the epithelial cell of the distal tubule in a parr (*d*); endocytosis zone and a portion of the brush border of type II epithelial cells in the proximal tubule in a spawner (*e*); basal part of the epithelial cell of the distal tubule in a smolt (*f*); apical part of the epithelial cell of the distal tubule in a smolt (*g*), part of the distal tubule in a spawner (*h*). Basement membrane (1), brush border (2), nucleus (3), lobed cytoplasmic process (4), epithelial cell of the proximal tubule (5), epithelial cell of the distal tubule (6), heterochromatin (7), mitochondria (8), smooth endoplasmic reticulum (9), vesicle (10).

Draft

Table 1. Outer diameters (μm) of the main structural units of trunk kidney and renal interstitium proportion (%) in brown trout *S. trutta*. Data are shown as mean $\pm$ SEM, n – number of individuals. Different letter superscripts within rows indicate statistically significant differences among the ontogenetic stages (Kruskal–Wallis ANOVA with post hoc Wilcoxon–Mann–Whitney test).

Parameter	Parr, $n=5$	Smolt, $n=4$	Spawner, $n=3$
Renal corpuscle, μm	70.58 ± 2.13^b	56.49 ± 3.81^a	69.24 ± 2.05^b
Proximal tubule, μm	56.97 ± 1.82	50.71 ± 3.11	54.31 ± 1.34
Distal tubule, μm	61.88 ± 2.58^b	47.48 ± 3.35^a	54.29 ± 1.45^a
Blood vessel, μm	25.84 ± 2.96	24.04 ± 2.39	29.49 ± 3.32
Interstitium proportion, %	51.55 ± 3.72	44.41 ± 1.98	51.21 ± 1.90

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Table 2. Morphometric parameters of leukocytes (CS = cell size; NS = nucleus size; MS = mitochondrion size; NM = number of mitochondria on a cell section; GS = granule size; NG = number of granules on a cell section) in the trunk kidney of parr (P), smolt (Sm) and spawning (Sp) brown trout *S. trutta*. Data are given as mean $\pm$ SEM, n – number of individuals; n.a. = not applicable. Different letter superscripts within columns indicate statistically significant differences among the ontogeny stages (Kruskal–Wallis ANOVA with post hoc Wilcoxon–Mann–Whitney test).

Leukocyte	Stage/ n	CS, μm^2	NS, μm^2	MS, μm^2	NM (min-max)	GS, μm^2	NG (min-max)
Lymphocyte	P/5	17.4 $\pm$ 3.42	10.6 $\pm$ 1.74	0.17 $\pm$ 0.05 ^b	1.00 $\pm$ 0.32 ^a (0-2)	n.a.	n.a.
	Sm/4	31.8 $\pm$ 10.5	17.5 $\pm$ 5.93	0.33 $\pm$ 0.05 ^a	4.25 $\pm$ 0.25 ^b (4-5)	n.a.	n.a.
	Sp/3	18.2 $\pm$ 1.71	9.87 $\pm$ 0.89	0.14 $\pm$ 0.03 ^b	2.00 $\pm$ 0.58 ^a (1-3)	n.a.	n.a.
Plasma cell	P/5	62.6 $\pm$ 10.5	30.2 $\pm$ 5.73	0.24 $\pm$ 0.04 ^b	4.33 $\pm$ 0.88 (3-6)	n.a.	n.a.
	Sm/4	91.2 $\pm$ 14.3 ^a	42.4 $\pm$ 16.7 ^a	0.72 $\pm$ 0.08 ^a	7.33 $\pm$ 0.67 (6-8)	n.a.	n.a.
	Sp/3	45.3 $\pm$ 10.1 ^b	14.0 $\pm$ 3.44 ^b	0.16 $\pm$ 0.01 ^b	3.50 $\pm$ 0.65 (2-5)	n.a.	n.a.
Macrophage	P/5	107 $\pm$ 9.27	18.3 $\pm$ 2.92	0.25 $\pm$ 0.02 ^b	3.75 $\pm$ 1.25 (1-7)	n.a.	n.a.
	Sm/4	124 $\pm$ 21.4	18.1 $\pm$ 4.20	0.63 $\pm$ 0.15 ^a	4.75 $\pm$ 0.48 (4-6)	n.a.	n.a.
	Sp/3	54.7 $\pm$ 5.11	6.37 $\pm$ 2.24	0.09 $\pm$ 0.01 ^b	3.80 $\pm$ 0.73 (2-6)	n.a.	n.a.
Neutrophil	P/5	54.2 $\pm$ 4.51	17.9 $\pm$ 2.29 ^b	0.09 $\pm$ 0.01 ^b	5.00 $\pm$ 1.05 (2-9)	0.12 $\pm$ 0.01 ^b	9.50 $\pm$ 1.31 ^b (6-15)
	Sm/4	57.5 $\pm$ 6.02	4.10 $\pm$ 0.46 ^a	0.16 $\pm$ 0.03 ^a	5.00 $\pm$ 0.52 (4-7)	0.09 $\pm$ 0.02 ^a	15.0 $\pm$ 3.19 ^a (10-24)
	Sp/3	46.0 $\pm$ 6.33	11.0 $\pm$ 2.34 ^b	0.07 $\pm$ 0.01 ^b	3.40 $\pm$ 0.24 (3-4)	0.12 $\pm$ 0.01 ^b	8.17 $\pm$ 1.38 ^b (5-13)
Eosinophil	P/5	42.2 $\pm$ 7.58	9.35 $\pm$ 2.03	0.19 $\pm$ 0.05	2.40 $\pm$ 0.24 ^a (2-3)	0.38 $\pm$ 0.05 ^b	27.7 $\pm$ 7.49 ^b (15-48)
	Sm/4	64.8 $\pm$ 14.0	8.43 $\pm$ 2.00	0.20 $\pm$ 0.01	5.25 $\pm$ 0.63 ^b (4-7)	0.19 $\pm$ 0.01 ^a	117 $\pm$ 21.9 ^a (55-157)
	Sp/3	48.5 $\pm$ 4.10	11.7 $\pm$ 1.64	0.14 $\pm$ 0.02	1.75 $\pm$ 0.63 ^a (0-3)	0.82 $\pm$ 0.29 ^b	20.7 $\pm$ 0.67 ^b (20-22)

Table 3. Morphometric parameters of cells with radially-arranged vesicles and chloride cells (CS = cell size; NS = nucleus size; MS = mitochondrion size; NM = number of mitochondria on a cell section; VS = vesicle size; NV = number of vesicles on a cell section) in the trunk kidney of parr, smolt and spawning brown trout *S. trutta*. Data are given as mean $\pm$ SEM, n – number of individuals; n.f. = not found; n.a. = not applicable.

Parameter	Cells with radially arranged vesicles			Chloride cell		
	Parr, $n=5$	Smolt, $n=4$	Spawner, $n=3$	Parr, $n=5$	Smolt, $n=4$	Spawner, $n=3$
CS, μm^2	38.8 $\pm$ 1.20	44.7 $\pm$ 3.52	28.6 $\pm$ 4.25	n.f.	76.6 $\pm$ 10.9	66.9 $\pm$ 5.74
NS, μm^2	17.1 $\pm$ 1.66	11.08 $\pm$ 2.20	13.0 $\pm$ 2.33	n.f.	7.42 $\pm$ 0.74	7.36 $\pm$ 0.39
MS, μm^2	0.14 $\pm$ 0.02	0.18 $\pm$ 0.02	0.10 $\pm$ 0.02	n.f.	0.46 $\pm$ 0.15	0.46 $\pm$ 0.10
NM	4.00 $\pm$ 0.58	3.67 $\pm$ 0.67	3.00 $\pm$ 1.53	n.f.	25.3 $\pm$ 0.33 ^a	10.3 $\pm$ 0.67 ^b
(min-max)	(3-5)	(3-5)	(1-6)		(25-26)	(9-11)
VS, μm^2	0.08 $\pm$ 0.02 ^b	0.16 $\pm$ 0.07 ^a	0.09 $\pm$ 0.05 ^b	n.a.	n.a.	n.a.
NV	22.3 $\pm$ 0.58	28.7 $\pm$ 1.47	24.0 $\pm$ 9.24	n.a.	n.a.	n.a.
(min-max)	(21-23)	(27-31)	(8-40)			

Table 4. Morphometric parameters of the proximal tubule epithelial cells (CS = cell size; NS = nucleus size; MS = mitochondrion size; NM = number of mitochondria on a cell section; SGS = secretory granule size; NSG = number of secretory granules on a cell section; EZS = endocytosis zone size; BBS = brush border size; CD = cilium diameter; MD = microvillus diameter) in the trunk kidney of parr, smolt and spawning brown trout *S. trutta*. Data are given as mean ± SEM, *n* – number of individuals; n.a. = not applicable. Different letter superscripts within rows indicate statistically significant differences among the ontogeny stages (Kruskal–Wallis ANOVA with post hoc Wilcoxon–Mann–Whitney test).

Parameter	Type I epithelial cells of the proximal tubule				Type II epithelial cells of the proximal tubule		
	Parr, <i>n</i> =5	Smolt, <i>n</i> =4	Spawner, <i>n</i> =3		Parr, <i>n</i> =5	Smolt, <i>n</i> =4	Spawner, <i>n</i> =3
CS, μm ²	89.8 ± 14.3 ^b	54.1 ± 7.07 ^a	63.2 ± 1.72 ^b	Basal	89.1 ± 11.1 ^b	64.1 ± 16.3 ^a	73.3 ± 6.72 ^b
NS, μm ²	30.4 ± 3.97	24.6 ± 4.32	28.5 ± 2.60		34.4 ± 3.41	28.0 ± 4.93	31.1 ± 1.90
MS, μm ²	0.19 ± 0.05 ^b	0.47 ± 0.20 ^a	0.29 ± 0.09 ^b		0.18 ± 0.03 ^b	0.23 ± 0.02 ^a	0.19 ± 0.05 ^b
				Apical			
NM	18.2 ± 4.07 ^b	35.5 ± 3.65 ^a	15.7 ± 1.70 ^b		0.13 ± 0.02 ^b	0.17 ± 0.03 ^a	0.08 ± 0.01 ^b
(min-max)	(11-29)	(18-41)	(11-19)		26.0 ± 1.51 ^b	86.7 ± 10.3 ^a	18.7 ± 2.84 ^b
SGS, μm ²	0.61 ± 0.08 ^b	1.67 ± 0.33 ^a	0.46 ± 0.12 ^b		n.a.	n.a.	n.a.
NSG	16.2 ± 2.56	11.0 ± 1.84	8.00 ± 0.95		n.a.	n.a.	n.a.
(min-max)	(6-30)	(3-19)	(5-10)				
EZS, μm	3.83 ± 0.47	3.51 ± 0.35	3.53 ± 0.31		2.00 ± 0.15	2.75 ± 0.16	2.27 ± 0.14
BBS, μm	2.66 ± 0.16	2.48 ± 0.18	3.13 ± 0.29		2.01 ± 0.39	2.02 ± 0.25	1.70 ± 0.20
CD, μm	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00		0.20 ± 0.00	0.19 ± 0.00	0.20 ± 0.00
MD, μm	0.09 ± 0.00	0.11 ± 0.00	0.11 ± 0.00		0.10 ± 0.00 ^a	0.16 ± 0.01 ^b	0.18 ± 0.03 ^b

Table 5. Morphometric parameters of the distal tubule epithelial cells (CS = cell size; NS = nucleus size; MS = mitochondrion size; NM = number of mitochondria on a cell section) in the trunk kidney of parr, smolt and spawning brown trout *S. trutta*. Data are given as mean $\pm$ SEM, n – number of individuals. Different letter superscripts within rows indicate statistically significant differences among the ontogeny stages (Kruskal–Wallis ANOVA with post hoc Wilcoxon–Mann–Whitney test).

Parameter	Parr, $n=5$	Smolt, $n=4$	Spawner, $n=3$
CS, μm^2	127 ± 0.12	122 ± 5.59	122 ± 4.77
NS, μm^2	28.1 ± 1.74	23.6 ± 2.25	32.2 ± 1.91
MS, μm^2			
Basal	0.39 ± 0.05	0.52 ± 0.05	0.43 ± 0.18
Apical	0.29 ± 0.09^b	0.68 ± 0.19^a	0.17 ± 0.02^b
NM	35.2 ± 2.10^b	80.0 ± 9.08^a	30.75 ± 4.84^b
(min-max)	(25-50)	(67-106)	(22-41)

Figure 1. Location of sampling sites on the Luga River (1) and the Solka River (2). Figure was created using SAS.Planet version 200606 (<http://www.sasgis.org>) and assembled from the following data sources: base map (Al-Hamdani et al. 2007); contour lines from NOAA National Geophysical Data Center ETOPO1 (Amante and Eakins 2009); shoreline (Harff et al. 2011).

204x74mm (300 x 300 DPI)

Figure 2. Microanatomy of the trunk kidney in parr (a, b), smolt (c) and spawning (d) brown trout *S. trutta*. Main structural units: blood vessel (1), renal interstitium (2), renal corpuscle (3), proximal tubule (4), distal tubule (5).

Figure 3. Ultrastructure of agranulocytes (mononuclear leukocytes) in the renal interstitium of the brown trout *S. trutta*. Lymphocyte with a spherical nucleus in a parr (a); lymphocyte with a lobed nucleus in a smolt (b); lymphocyte with a segmented nucleus in a spawner (c); plasma cell in a parr (d); plasma cell in a smolt (e); plasma cell in a spawner (f); cytoplasm of a macrophage in a parr (g); macrophage in a spawner (h), melano-macrophage centre in a smolt (i). Units: nucleus (1), heterochromatin (2), mitochondria (3), rough endoplasmic reticulum (4), phagosomes (5), melanin granules (6), granulocyte aggregate (7).

Figure 4. Ultrastructure of granulocytes (polymorphonuclear leukocytes) in the renal interstitium of the brown trout *S. trutta*. Neutrophil with a horseshoe-shaped nucleus in a parr (a); specific granules of neutrophil in a parr (b); neutrophil with a segmented nucleus in a spawner (c); eosinophil with a spherical nucleus in a parr (d); segmented eosinophil with atypical granules in a spawner (e); band eosinophil in a smolt (f). Cell units: nucleus (1), heterochromatin (2), mitochondria (3), rough endoplasmic reticulum (4), specific granules (5).

Figure 5. Ultrastructure of cells with radially arranged vesicles and chloride cells in the renal interstitium of the brown trout *S. trutta*. Cell with radially arranged vesicles in a parr (a); cytoplasm of a cell with radially arranged vesicles in a smolt (b); cell with radially arranged vesicles in a spawner (c); cytoplasm of a chloride cell in a smolt (d); chloride cell in a smolt (e); cytoplasm of a chloride cell in a spawner (f). Cell units: nucleus (1), heterochromatin (2), mitochondria (3), radially arranged vesicles (4), cell centre (5), rough endoplasmic reticulum (6), tubular endoplasmic reticulum (7), electron-dense granules (8).

Figure 6. Ultrastructure of the renal corpuscle end proximal tubule in the trunk kidney of brown trout *S. trutta*. Part of renal corpuscle in a parr (a); podocyte in a spawner (b); part of the proximal tubule in a parr (c); endocytosis zone and a portion of the brush border in a parr (d); basal part of type I epithelial cell of the proximal tubule in a parr (e); basal part of type I epithelial cell of the proximal tubule in a smolt (f); basal part of the proximal tubule with type I epithelial cells in a spawner (g); ciliated epithelial cell in a spawner (h); cilia (i). Cell units: urinary space (1), basement membrane (2), squamous epithelial cell (3), podocytes (4), nucleus (5), heterochromatin (6), endothelial cell (7), capillary (8), brush border (9), epithelial cell of proximal tubule (10), mitochondria (11), smooth endoplasmic reticulum (12), endocytosis zone (13), secretory granules (14), microvilli (15), cilia (16), tubular endoplasmic reticulum (17), vesicle (18).

Figure 7. Ultrastructure of the proximal and distal tubule in the trunk kidney of brown trout *S. trutta*. Part of the proximal tubule with type II epithelial cells in a parr (a); part of the distal tubule in a parr (b); basal part of II type epithelial cell of the proximal tubule in a parr (c); basal part of the epithelial cell of the distal tubule in a parr (d); endocytosis zone and a portion of the brush border of type II epithelial cells in the proximal tubule in a spawner (e); basal part of the epithelial cell of the distal tubule in a smolt (f); apical part of the epithelial cell of the distal tubule in a smolt (g), part of the distal tubule in a spawner (h). Basement membrane (1), brush border (2), nucleus (3), lobed cytoplasmic process (4), epithelial cell of the proximal tubule (5), epithelial cell of the distal tubule (6), heterochromatin (7), mitochondria (8), smooth endoplasmic reticulum (9), vesicle (10).