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Fish anatomy

STRUCTURAL CHANGES IN SEA WATER-ADAPTED RAINBOW TROUT (ONCORHYNCHUS MYKISS WALB.) GILLS

ZMIANY STRUKTURALNE W SKRZELACH PSTRĄGA TĘCZOWEGO (ONCORHYNCHUS MYKISS WALB.) PRZENIESIONEGO DO WODY SŁONAWEJ

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Changes in juvenile rainbow trout (Oncorhynchus mykiss Walb.) gills at different times after transfer to a brackish water (4–7\%\textsubscript{o}) culture in the Puck Bay were followed. Total gill tissue mounts as well as thin and ultra-thin sections were examined. Acute inflammation of the gill tissue, observed initially, receded after 2 weeks of adaptation. After 2 months in the culture, the gill structure was transformed, the transformations involving, i.a. increase in the activity and number of chloride cells and increase in the gill epithelium intercellular spaces.

INTRODUCTION

Salmonids, including rainbow trout (Oncorhynchus mykiss Walb.), have been for a relatively long time released into estuarine and marine waters, the release always inciting a great interest on the part of scientists (Meyer 1939; Kulmatycki 1940). Attempts to stock the Baltic’s inshore waters with rainbow trout or to intensively cage culture the species there have been made as well. However, those attempts have not always been successful and are still viewed as a commercial-scale experiment, sometimes a very costly one (Trzebiatowski 1979; Bartel 1981; Wawrzyniak 1986; Wiktor 1986).

The descending salmonids undergo, under natural conditions, a complex and long-lasting process of smolting. That natural process has not been adequately accounted for during stocking operations and in cage cultures in the Baltic. It turned out, however, that an
abrupt habitat change, from fresh- to seawater, does leave its mark in the fish system. The consequences involve both short- and long-term alterations which occur primarily in those organs responsible for osmotic regulation; life processes may be disturbed as well (Cykowska 1977, 1978; Lubin et al. 1989; Szwal 1991). Changes resulting from environmental pollution may be superimposed on those caused by the habitat change (Dąbrowska 1974; Coleman et al. 1977; Wawrzyniak and Grawiński 1988).

Gills belong to the organs in which alterations brought about by adaptive processes related to change of habitat, from fresh- to seawater, are pronounced. For this reason, it was the gills that were observed in the first place when studying changes induced by an abrupt transfer and seawater adaptation of rainbow trout (Wawrzyniak et al. 1999).

MATERIAL AND METHODS

The study involved pond-cultured rainbow trout (*Oncorhynchus mykiss* Walb.) individuals which, when aged 1+, were transferred to a cage culture situated in the brackish (4–7%) Puck Bay.

Branchial lamellae slides made prior to transfer and those obtained after 10 min., 1, 3, 6, 12, 24, and 48 h as well as after 2 weeks of seawater adaptation were examined under a compound microscope. Microstructural changes visible in the rainbow trout gill lamellae after 2 months in the cage culture were analysed as well, using a TESLA 242E electron microscope.

Gill tissue samples were collected from the mid-part of the first gill arch of freshly narcotised fish. The samples were fixed with 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 2 h at 4°C and treated with 1% osmium tetroxide (Sjöstrand 1967; Robinson et al. 1987; Cieciura 1989). The Westopal-embedded mounts, cut into ultra-thin (300–600 Å) and semi-thin sections, were treated as described by Reynolds (1963); the semi-thin sections were May-Grünwald stained. The terminology proposed by Morgan and Tovell (1973), Morgan (1974), Hughes (1977), Rajbanshi (1977), Brandt and Eble (1979), and Morrison (1979) was used when describing gill photomicrographs and TEM photomicrographs.

RESULTS

Adaptive processes were manifested on the total gill tissue mounts examined as changes on the branchial tissue surface (mucus secretion, hyperaemia, and swelling of gill lamellae). Those changes, appearing soon after transfer, were becoming more profound with time. In addition, the semi-thin sections revealed some biostuctural changes (activity of mucus and chloride cells) within the branchial epithelium (Figs. 1, 2).
As early as 10 min. after transfer, gill lamellae tips turned anaemic. The lamella epithelium showed cytoplasm thinning in a few places as well as enlarged nuclei. After 60 min., branchial gill anaemia intensified and a mucus film (a thin mucopolysaccharide layer visible on ultra-thin TEM-examined sections) appeared on the surface of gill lamellae.

Within 3–6 h from transfer, localised lamella swelling and congestions began to appear. Localised haemorrhages in lamella capillaries were observed. Activity of mucus cells intensified, the secretion covering the entire surface of the branchial lamellae. Intercellular spaces within the lamella epithelium increased. The appearance of the chloride cells was indicative of their increasing activity (Fig. 2).

After 12 h of adaptation, the entire gill lamellae turned congested. They were locally swollen and showed localised blood cell aggregations. The chloride cells, situated basally in the lamellae, became swollen as well. Most epithelial cells on the swollen branchial lamellae appeared much larger (Fig. 2) than those in the control (Fig. 1). The process was clearly intensified after 24 h. Some of the swollen epithelial cells broke down, their cytoplasm spilling out of the lamellae. Activity of the mucus cells diminished, whereby the mucus layer covering the gills disappeared. The lumen of the branchial lamella marginal canals was clearly enlarged, compared to the control (Fig. 3) and filled with blood cells (Fig. 4).

After 48 h, the gill lamellae (with localised petechiae) were less congested. Microscope slides showed enlarged lumen of the capillaries, damaged supporting cells, and large aggregations of blood cells.

The acute inflammation symptoms were observed to disappear after 2 weeks of adaptation, while atrophic changes in the mucus cells, swelling of the epithelial cells, and increased activity of the chloride cells ensued.

Examinations of ultra-thin sections of branchial lamellae, made after 2 months of rainbow trout adaptation to the Puck Bay habitat, showed the lamellae to have undergone numerous structural alterations, compared to the control, i.e. the lamellae collected from the fish kept in fresh water. The latter were usually thin, whereby the water-blood barrier was thin as well, thus facilitating diffusion of gases (Figs. 1, 3).

The external cells of the gill lamellae, active in respiration, were—along with their nuclei—clearly flattened and tightly interconnected by long intercellular coupling complexes. The cell surfaces were covered by well-developed micro-ridges. On the side of the lamella opposite to that housing respiratory cells there were cells which Morgan and Tovell (1973) described as “dark”, Morrison (1979) identified as “dense”, and Shen and Leatherland (1978) referred to as “mitochondria-rich”. Their abundant mitochondria were usually situated in the basal part of a cell. The mitochondria-rich cells featured large nuclei and were supplied, in their apical part, with a dense network of endoplasmic reticulum and
pinocytal bodies. Micro-ridges on the surface were visible as well (Figs. 5, 8). Those large, granular cytoplasm-filled cells protruded somewhat from the branchial lamella surface.

In addition, the presence of intercellular spaces between the outer and inner epithelial cell layers was observed. The intercellular spaces are typical of structures responsible for ionic exchange. They are relatively small in freshwater fish in which they sometimes appear as gaps and do not affect the shape and thickness of the branchial lamellae.

The gill biostructure in those individuals adapted to seawater for 2 months showed considerable changes. Certain characteristic differences, relative to a typical pattern of gill organisation (Bird and Eble 1974; Morrison 1974), appeared. The differences were primarily due to the loss of clarity and ordering of the structures examined. This is particularly relevant with respect to the enlarged lumen of the marginal canal, general gill hyperaemia, epithelial cell swelling, and increased activity and size of the chloride cells proper. The intercellular spaces in the epithelium underwent the most conspicuous transformations (Figs. 6, 7). The spaces became broadened, thereby rendering the entire gill lamellae thicker. The intercellular spaces were observed to contain granules of variable size and infiltrating leukocytes (Fig. 7). The endoplasmic reticulum became enlarged as well, the number of pinocytal bodies in the mitochondria-rich cells increasing (Fig. 8). The increased volume of the gill lamellae resulted in a clear and substantial contraction of the epithelial cell coupling complexes, accompanied by a reduction in the number and height of the micro-ridges on the cell surface; the micro-ridges may occasionally disappear altogether. The adaptive process-related changes involved also the interior of branchial lamellae via the activity of the lamella lining cells, broadening of the marginal canal and capillaries, and increase in the amount of blood accumulation in the sinuses (Figs. 2, 4, 6, 10).

The seawater-adapted fish showed increased secretory activity, followed by an increase in the number of the chloride cells proper, situated at the base of the gill lamellae (Figs. 2, 11, 12).

DISCUSSION

As shown by macroscopic, microscopic, and sub-microscopic observations, gill cells of rainbow trout provide a good material on which to follow processes related to the species' adaptation to seawater conditions. An abrupt transfer from fresh to brackish (4–7%) water results in a shock the effects of which are visible in gills as early as after 10–60 min. Within the initial 48 h following the transfer, the changes intensify and are manifested as swelling, congestions, frequent cell damage, and/or irreversible cell alterations. To counteract to shock, the fish’s mucus cells intensify their activity. A thick mucus layer covers the surface of branchial lamellae. This in turn leaves its mark the
Fig. 1. Photomicrograph of a semi-thin gill lamella section of freshwater-kept rainbow trout. Gill lamellae thin (phase contrast; × 360)

Fig. 2. A cross-section through a gill lamella fragment from a seawater-adapted rainbow trout, with clear signs of lamella thickening caused by epithelial cell vacuolisation. Chloride cells enlarged, at the stage of secretory activity (× 360)
Fig. 3. Photomicrograph of a freshwater-kept rainbow trout gill lamellae: lamellae thin, with body blood uniformly within sinuses and the marginal canal (× 400)

Fig. 4. Functional and dystrophic changes within seawater-adapted fish gill lamellae. Congestions, blood-filled marginal canal, and damaged epithelial cells visible (× 400)
Fig. 5. TEM photomicrograph of a cross-section of freshwater-kept rainbow trout gill lamella. Clearly flattened respiratory epithelium cells visible to the supporting cell. A protruding large, mitochondria-rich cell involved in ionic regulation visible to the right (× 9000)

Fig. 6. Blow-up of a basal fragment of a mitochondria-rich cell, in a close contact with the gill lamella sinus basal membrane; respiratory gaps indicated by arrows (× 44 000)
Fig. 7. A fragment of a seawater-adapted rainbow trout gill lamella. Intercellular spaces greatly enlarged, with microbodies and granules (× 9500)

Fig. 8. TEM photomicrograph of a cross-section of freshwater-kept rainbow trout gill lamella, with enlarged intercellular spaces (× 22000)
Fig. 9. Infiltration of leukocytes to intercellular spaces in the seawater-adapted rainbow trout gill lamella epithelium ($\times$ 9000)

Fig. 10. A fragment of a seawater-kept rainbow trout gill lamella mitochondria-rich cell with profuse endoplasmic reticulum, pinocytal bodies, and granules ($\times$ 22 000)
Fig. 11. A fragment of a freshwater-kept rainbow trout gill lamella mitochondria-rich cell; a long, overlapping fragment of the coupling complex of two respiratory cells with well-developed micro-ridges (× 22 000)

Fig. 12. TEM photomicrograph showing a short fragment of the coupling complex of external gill lamellae cells of a seawater-adapted rainbow trout (× 44 000)
Fig. 13. TEM photomicrograph of an area housing chloride cells proper, situated between gill lamellae of a seawater-adapted rainbow trout (× 9000)

Fig. 14. Blow-up of a fragment of the apical of a seawater-adapted rainbow trout chloride cell at intensive secretion phase (× 44000)
# ABBREVIATIONS

| Abbreviation | Description          |
|--------------|----------------------|
| BM          | basement membrane    |
| BS          | blood space          |
| CCH         | chloride cell        |
| IEP         | inter epithelium     |
| M           | mitochondria         |
| MB          | microbody            |
| MC          | marginal canal       |
| MV          | microvilli           |
| N           | nucleus              |
| OEP         | outer epithelium     |
| PC          | pilar cell           |
| R           | ridge                |
| SE          | secondary lamella    |
| X           | non tissue           |
Structural changes in seawater adapted rainbow trout gills

behaviour and condition of the fish affected (Ultsch and Gros 1979; Handy and Eddy 1989). Initially after transfer to brackish water, the fish growth is retarded, weight reduction being occasionally recorded (Winnicki et al. 1981; Wawrzyniak 1986, 1998).

During the first two weeks after transfer, the adaptive changes intensify and become more persistent; on the other hand, the osmotic shock effects recede. No swelling or hyperaemia are observed any more. The gill mucus coating disappears as early as after two days.

The changes in gills of the seawater-adapted fish, recorded in this study, confirm observations reported by other authors (Morgan and Tovell 1973; Cykowska 1977, 1978; Morrison 1979). Activity and size of the chloride cells increase, as does their amount. The activity increase is indicated by an increase in the number of mitochondria and pinocytal vesicles as well as by enhancement of the smooth endoplasmic reticulum and microvilli-topped cytoplasmic thickenings in the “mitochondria-rich” cells.

According to Wright (1974), aggregation of mitochondria in the gill epithelium cells is indicative of intensified transport of dissolved substances in the area affected, which is also associated with increased intracellular spaces playing a significant role in buffering the osmotic shock. The microbodies observed may participate in ionic regulation. Infiltration of leukocytes from capillaries into the intercellular spaces of the epithelium may be an evidence of inflammations caused by osmotic shock-depressed immunological response to pathogens (Dąbrowska 1974; Wawrzyniak and Grawiński 1988). This could be indirectly confirmed by the presence of parasitic amoebae in the branchial lamella sinuses (Radziun, pers. obs.). A similar swelling of the rainbow trout branchial lamellae and increased intracellular spaces was observed (Karlsson-Norr gren et al. 1985) under exposure to toxic substances (cadmium).

During salt-water adaptation, extensive damage of the gill lamella surfaces due to swelling is prevented by contraction of the coupling complexes and by levelling off of the micro-ridges.

As a phylogenetically young species, the rainbow trout whose varieties (steelhead) stay in salt water for prolonged periods of time, is physiologically flexible and has a high tolerance to increased habitat salinity. However, to avoid the osmotic shock manifested as acute inflammations, certain adaptive procedures have to be followed.

To sum up, it can be concluded that the rainbow trout gills are a good model on which to follow the course of seawater adaptation in fish.
CONCLUSIONS

1. The rainbow trout, a phylogenetically young species, is physiologically flexible and has a good tolerance to increased habitat salinity.

2. Abrupt transfer to seawater results in gill inflammations (swellings, congestions, cell damage), which recede almost completely within 2 weeks.

3. The picture of gills of the seawater adapted fish differs from that of the control by the presence of increased abundance of more active chloride cells, swollen cells, broadened intercellular spaces, and contracted coupling complexes, the changes aimed at buffering the osmotic shock incurred.

REFERENCES

Bartel R., 1981: Możliwości wysiedlania pstrąga tęczowego (Salmo gairdneri Rich.) do Bałtyku [Potentials and perspectives for the release of rainbow trout (Salmo gairdneri Rich.) in the Baltic]. DSc Thesis, Agricultural University in Kraków, Nr 81. (In Polish).

Bird D.J., A.F. Able, 1979: Cytology and polysaccharide cytochemistry of the gill of the American eel, Anguilla rostrata. Biol. Bull., 157: 104-111.

Cieciura L. (ed.), 1989: Techniki stosowane w mikroskopii elektronowej [Techniques of electron microscopy]. PWN, Warszawa. (In Polish).

Coleman R., Z. Yaron, Z. Ilan, 1977: An ultrastructural study of the mitochondria-rich ‘chloride’ cells from the gills of freshwater and seawater-adapted Tilapia aurea subjected to a pesticide. J. Fish Biol., 11: 589-594.

Cykowska C., 1977: Zmiany w mikrostrukturze skrzel i nerki pstrąga tęczowego (Salmo gairdneri Richardson) introdukowanego do morza [Changes in microstructure of gills and kidney of rainbow trout (Salmo gairdneri Richardson)]. PhD Thesis, Agricultural University of Szczecin. (In Polish).

Cykowska C., 1978: Changes in microstructure of gills in rainbow trout, Salmo gairdneri Richardson, adapted to sea water conditions. Acta Ichthyol. Piscat., 8, 2: 59-76.

Dąbrowska H., 1974: Próba oceny stanu zdrowotnego ryb w rzce Łynie i Wałszy na tle ich zanieczyszczenia [An attempt to evaluate the state of health of fish from the Łyna and Wałsza rivers in connection to their pollution]. Przegl. Zool., 18, 3: 300-395.

Handy R.D., F.B. Eddy, 1989: Surface absorption of aluminium by gill tissue and body mucus of rainbow trout, Salmo gairdneri, at the onset of episodic exposure. J. Fish Biol., 34: 865-874.

Hughes G.M., 1979: Scanning electron microscopy of the respiratory surfaces of trout gills. J. Zool., London, 187: 443-453.

Karlsson-Norrgren L., P. Runn, C. Haux, L. Förlin, 1985: Cadmium-induced changes in gill morphology of zebrafish, Brachydanio rerio (Hamilton–Buchanan), and rainbow trout, Salmo gairdneri Richardson. J. Fish Biol., 27: 81-95.

Kulmatycki W.I., 1940: Über das Wachstum und die Wanderungen in der Ostsee ausgesetzter Forellen. Verh. Int. Ver. Theor. Angew. Limnol., 9: 267-275.
Lubin R.T., A.W. Rourke, T.M. Bradley, 1989: Ultrastructural alterations in branchial chloride cells of Atlantic salmon, Salmo salar, during parr–smolt transformation and early development in sea water. J. Fish Biol., 34: 259–272.

Meyer P.F., 1939: Aussetzungen von Regenbogenforellen (Salmo irideus Gibb.) in der Ostsee. Ber. DWK Meerforsch., NF, 9, 2: 318–322.

Morgan M., 1974: Development of secondary lamellae of the gills of the trout, Salmo gairdneri (Richardson). Cell. Tiss. Res., 151: 509–523.

Morgan M., P.W.A. Tovell, 1973: The structure of the gill of the trout, Salmo gairdneri (Richardson). Z. Zellforsch., 142: 147–162.

Morrison C.M., 1979: A dense cell in the epithelium of the gill lamellae of the brook trout, Salvelinus fontinalis (Mitchill). J. Fish Biol., 15: 601–605.

Rajbanshi V.K., 1977: The architecture of the gill surface of the catfish, Heteropneustes fossilis (Bloch): SEM study. J. Fish Biol., 10: 325–329.

Reynolds E.S., 1963: The use of lead citrate and high pH as an electron-opaque stain in electron microscopy. J. Cell. Biol., 17: 208–212.

Robinson D.G., U. Ehlers, R. Harken, B. Hermenn, F. Mayer, F.W. Schürmann, 1987: Methods of preparation for electron microscopy. Springer Verlag, New York.

Shen A.C.Y., J.F. Leatherland, 1978: Structure of the yolksac epithelium and gills in the early developmental stages of rainbow trout (Salmo gairdneri) maintained in different ambient salinities. Env. Biol. Fish., 3, 4: 345–354.

Sjöstrand F.S., 1967: Electron microscopy of cells and tissue. Academic Press, New York–London.

Szwал T., 1991: Wpływ adaptacji płotka tęczowego (Salmo gairdneri Rich.) do wody morskiej na biostrukturu skrzel [Effects of seawater adaptation of rainbow trout (Salmo gairdneri Rich.) on gill biostructure]. MSc Thesis, Agricultural University of Szczecin. (In Polish).

Trzebiatowski R., 1973: Wstępne wyniki zarybiania znakowanym płotkiem Zalewu Szczecińskiego i wód przybrzeżnych Bałtyku [Preliminary results of stocking the Szczecin Lagoon and Baltic inshore waters with tagged rainbow trout]. Zesz. Nauk. AR w Szczecinie, No 40: 480–505. (In Polish).

Trzebiatowski R., 1979: Teoretyczne i praktyczne przesłanki możliwości wsiedlania płotka tęczowego (Salmo gairdneri Rich.) do wód przybrzeżnych Bałtyku [Theoretical and practical premises of stocking the Baltic coastal waters with rainbow trout (Salmo gairdneri Rich.).] DSc Thesis, Zesz. Nauk. Akademii Rolniczej w Szczecinie, ser. Rozprawy, nr 61. (In Polish).

Ultsch G.R. G. Gros, 1979: Mucus as a diffusion barrier to oxygen: possible role in $O_2$ uptake at low pH in carp (Cyprinus carpio) gills. Comp. Biochem. Physiol., 62A: 685–689.

Wawrzyniak W., 1986: Wpływ wybranych czynników zewnętrznych na zmiany masy jednostkowej płotka tęczowego (Salmo gairdneri Rich.) z hodowli morskiej w świetle analizy matematycznej [Effects of selected environmental factors on changes in individual weight of seawater-cultured rainbow trout (Salmo gairdneri Rich.) in the light of mathematical analysis]. PhD Thesis, Agricultural University of Szczecin. (In Polish).

Wawrzyniak W., 1998: Rozważania nad istotą wzrostu masy organizmu ryby na tle przykładowych modeli płotka tęczowego (Oncorhynchus mykiss Walb.) [The nature of organismal weight growth in fish, as determined from models of rainbow trout (Oncorhynchus mykiss Walb.).] DSc Thesis, Uniwersytet Szczeciński, Rozprawy i Studia, 272. (In Polish).

Wawrzyniak W., E. Grawiński, 1988: Próba określenia przyczyny powstawania zewnętrznych zmian anatomopathologicznych u ryb bałtyckich [An attempt to determine the cause of external anatomopathological changes in Baltic fish]. Biuletyn Inform., CLPR Gdynia, Nr 1: 5–18. (In Polish).
Wawrzyniak W., K. Radziun, A. Sobociński, 1999: Problem adaptacji pstrąga tęczowego (Oncorhynchus mykiss Walb.) do środowiska zasolonego, a zmiany strukturalne w skrzelach i jelicie środowiskowym [The problem of seawater adaptation of rainbow trout (Oncorhynchus mykiss Walb.) versus structural changes in gills and intestine]. XVII Zjazd PTZool. „Bioróżnorodność, zasoby i potrzeby ochrony fauny Polski” 20–23.09.1999 r., Słupsk [The 17th Conference of the Polish Zoological Society, “Biodiversity, resources, and needs in protection of the Polish fauna”, 20-23 September 1999, Słupsk]: 288–289. (In Polish).

Witkori J., 1986: Sadzowa hodowla pstrąga tęczowego w Zatoce Puckiej [Cage culture of rainbow trout in the Puck Bay]. Studia i Materiały MIR, 54 B. (In Polish).

Winnicki A., L. Tomasik, K. Radziun, W. Wawrzyniak, B. Walczak, A. Sobociński, 1981: Badania procesów adaptacyjnych u narybku pstrąga tęczowego pod kątem opracowania optymalnej metody wsiadania go do wody słonawej w warunkach hodowlí w sadzach pływających PPIUR „Szkuner” [Studies on adaptive processes in rainbow trout juveniles, important for developing an optimal method for release to brackish water, under conditions of floating cage culture of the “Szkuner” Fishing Company]. Agricultural University of Szczecin, unpublished manuscript. (In Polish).

Wright D.E., 1974: Morphology of the gill epithelium of the lungfish, Lepidosiren paradoxa. Cell. Tiss. Res., 153: 365–381.

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