Distribution dynamics of Rodlet cells in the gills under pathological alterations

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Abstract. The article describes the study of Rodlet cells in gills of 15 fish species of the Cyprinidae family, 1 fish species from the Siluridae family, 2 fish species from the Percidae family, and 1 fish species from the Esocidae family, all caught in Zhaiyk River, Republic of Kazakhstan. Rodlet cells at the present stage of research are classified as the cells of nonspecific immune response in fish. Besides, they can be used as biomarkers to monitor the state of water reservoirs. Serious pathological changes in gills of the surveyed fish were revealed by histomorphological technique, indicating the pollution of water environment. An increased number of Rodlet cells was associated with morphological alterations in the gills. It was also noted that in the gills of all species Rodlet cells are at the different stages of differentiation. Data obtained support the issue of protective and adaptive functions of Rodlet cells under adverse environmental conditions.

1 Introduction

Rodlet cells (RCs) are rod cells considered as one of the leukocytes variety. At present, their main function is considered to be the protection from foreign bodies, which ensures the adaptation of fish to biotic and abiotic factors and immunity to parasitic invasions [1]. RCs are found in the epithelial tissues of various fish organs (spleen, thymus, kidneys, gonads, etc.) in freshwater and marine bony fish species [2].

This cell type was first described in 1892 by Thelohan [3]. For over than a hundred years, scientists had classified them as parasites (Laguesse, 1895; Bannister, 1966; Mayberry et al., 1979; Viehberger & Bielek, 1982; Bielek & Viehberger, 1983; Richards et al., 1994), however later this hypothesis was refuted. Nowadays it is agreed that this type of cells belongs to leukocytes - eosinophilic granulocytes [4, 5].

The morphological structure of Rodlet cells is currently being studied considering the differences among genera and species, stage of cell differentiation. But their functional role remains a matter of debate. However, most researchers agree [6] that Rodlet cells play an important role in the fish immune system and it is based on the release of their specific secretory granules, rods, due to the contractility of the fibrous layer of cells¹.

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In all vertebrate species, particularly in fish, the immune system can be divided into adaptive – specific system and innate – nonspecific system [7, 8, 9]. The innate immune system does not retain the memory of previous reactions; it is the first to respond to infection and disease [10]. Rodlet cells are one of the cellular components of fish’s innate immune system.

There is experimental evidence suggesting the potential use of Rodlet cells as biomarkers, regardless of their function [11]. The histopathological analysis is a sensitive indicator of pollution-causing stress in teleost, as their organs participate in the biotransformation of various active compounds that present in the aquatic environment [12-14]. In particular, the gills are at the center of toxicological research, due to the fact that they are the initial target of waterborne xenobiotics. Moreover, gills are extremely sensitive to them due to constant contact with the external environment. Furthermore, the complex gill structure contributes to a large accumulation of xenobiotics in tissues [15, 16].

Scientists have described four forms of Rodlet cells according to their life cycle phases [17]. In the first two phases, the cells are not surrounded by a fibrous membrane and have crystalline inclusions or are amorphous and granular. The next phase is the semi-shell stage, which was described in detail (Mattey, 1987). The last phases have strong evidence in addition to the fact that mature rods, with their fibrous cytoplasmic border, do indeed develop into fish tissue from a particular type of cell, which in turn does not contain a border.

Information on the dependence of Rodlet cell proliferation on the development of compensatory-adaptive and pathological processes is absent in the available literature. Meanwhile, this seems important to understand the process of adaptation of fish to environmental conditions. In addition, the study of Rodlet cells may be important for a better understanding of the immune system of teleost fish.

Thus, the aim of the current study is to examine the distribution of Rodlet cells in the gills of fish living in Zhaiyk River (Kazakhstan) at different stages depending on the intensity of the compensatory-adaptive and pathological reactions.

2 Materials and Methods

The samples of gills of 19 fish species (Ballerus sapa, Squalius cephalus, Blicca bjoerkna, Aspius aspius, Scardinius erythrophthalmus, Abramis brama, Rutilus rutilus, Chondrostoma nasus, Carassius Gibelio, Ballerus Ballerus, Pelecus cultratus, Leuciscus idus, Vimba vimba, Cyprinus carpio, Silurus Glanis, Esox Lucius, Perca fluviatilis, Sander volgensis) from 4 families (Cyprinidae, Siluridae, Esocidae и Percidae) caught in the Zhaiyk River in the summer and autumn of 2020 were used for histopathological study.

The material was collected with the support of the management and employees of the Atyrau and West Kazakhstan branches of the Research and Production Center for Fisheries (RPCF) at two stations near the city of Uralsk ("Kablytobe" and "Kushum") and three stations in the Atyrau city ("Tatarsk", "Dambinsk" and "12 square pre-mouth of river"). Tissue samples were taken directly at the place of the fish collection with further immediate fixation in 10% buffered formalin. All samples were labeled following all established rules. The material was delivered to the laboratory of the Research Institute of Biology and Biotechnology of al-Farabi Kazakh National University for further processing. In the Laboratory of animal morphology of the Research Institute of Problems of Biology and Biotechnology, biological samples of gills were taken for histological analysis. The material was processed by classical methods of microscopic technique (Romeis, 1953).

Histological slides were studied using a light trinocular microscope, Microoptix MX300T, with a Scopeimage 9.0 imaging system. Microphotography of the obtained...
sections was carried out at x100, x200, x400, x1000 magnifications. All stages of the work were carried out in a logical sequence with the least expenditure of time and faster material processing.

3 Results and discussion

As a result of histopathological examination, compensatory-adaptive and pathological reactions were revealed in all selected fish. Among the compensatory-adaptive reactions, we noted that there was the proliferation and hyperplasia of cells of the primary and secondary gill epithelium. Along with these pathological reactions were represented by edema, microcirculatory disorders, cartilage deformation, necrosis, and destruction of the gill epithelium (Figure 1).

The revealed disturbances are similar to the changes noted during the impact of pollutants on the fish organism (Roberts, 2012): the significant proliferation of the stratified non-keratinizing epithelium, which took the form of epithelial plates with atrophied lamellae, hyperplasia of the respiratory epithelium, hypertrophy of goblet cells, edema and desquamation of the respiratory epithelium at the tops of the lamellae, a sharp expansion of capillaries, stasis of formed elements in them, the appearance of aneurysms, significant deformation of the hyaline cartilage, which entailed an arcuate change in the filament.

The study of a sample of fish showed the presence of Rodlet cells in the gills of all studied fish species at the second, third, and fourth stages of development.

Rodlet cells at the first stage were not found by us during the histopathological examination.

At the second maturing stage (Figure 2, A), Rodlet cells were located in the primary gill epithelium closer to the cartilaginous base of the gill lamella and were oriented along the cartilage. Rodlet cells at this stage had an elongated oval shape, a granular cytoplasm filled with secretory granules characteristic of them, and a nucleus with a bright basophilic color located at the edge.

At the third transitional stage (Figure 2, B), we noted that Rodlet cells moved from the basal part of the epithelial layer to the surface layers of the epithelium and were located...
perpendicular to the cartilaginous base of the primary gill lamella, where the nucleus was facing the basal part. Rodlet cells had an elongated oval shape. The cell itself was surrounded by a distinct capsule. The cytoplasm contained rod-shaped secretory granules oriented along the cell and occupying most of it.

In the fourth stage, which is rupture, Rodlet cells were rounded and located in both the primary and secondary epithelium. In the secondary gill epithelium, they could be seen both on the apical part of the lamellae and in the interlamellar space. The cytoplasm of these Rodlet cells contained a basophilic nucleus located along the edge of the cell and was either brightly eosinophilic or lightly foamy (Figure 2, C, D), which is probably associated with the process of secretion release.

![Fig. 2. A) Rodlet cell at the 2nd transitional stage in asp, Aspius aspius. B) Rodlet cell at the 3rd mature stage in crucian carp, Carassius gibelio. C, D) Rodlet cell at the 4th stage of rupture in catfish, Silurus glanis. (H&E), x400](image)

Depending on the intensity of compensatory-adaptive and pathological reactions, we divided the fish into two groups, respectively.

In the group with a predominance of compensatory-adaptive processes, we observed the proliferation of the second and third stages of development of Rodlet cells. With the development of pathological processes, the number of cells at the fourth stage of development increased.

Thus, the results of the study indicate that there is a dependence of the proliferation of Rodlet cells on the development of compensatory-adaptive and pathological processes.

### 4 Conclusion

As a result of the research, it was shown that the majority of caught fish from 4 families (Cyprinidae, Siluridae, Percidae, Esocidae) had compensatory-adaptive (proliferation and hyperplasia of cells of the primary and secondary gill epithelium) and pathological (edema, microvasculature disturbance, cartilage deformation, necrosis and destruction of gill epithelium) reactions.
The presence of Rodlet cells in the gills of all studied fish species at the second, third, and fourth stages of development were shown.

The results of our study do not refute previous findings (Abd-Elh afeez, 2016; Walaa et al., 2018; Abd-Elh afeez, 2018) that gill Rodlet cells release their contents into the environment. Also, in combination with a strategic location around blood vessels, may play an important role in host defense.

Accumulation of Rodlet cells in places of tissue necrosis as a result of exposure to xenobiotics, from our perspective, indicates that these cells are involved in nonspecific protective reactions of the body to the action of environmental factors.

Since teleost fish are the most numerous groups of vertebrates on the planet, they are widely used as bioindicators in most aquatic ecosystems. Thus, Rodlet cells can be used as markers of pathological processes in teleost fish organs, in our case - gills, and are supposed to be part of a nonspecific immune response.

References

6. B. Sayyaf Dezfuli, F. Pironi, B. Maynard, E. Simoni, G. Bosi, Fish & Shellfish Immunology, 121, 516-534 (2022)