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Evaluation of Gill Histopathological Biomarkers in *Neogobius sp.* for Monitoring Freshwater Quality: A Case Study of the Veleka River, Bulgaria

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Abstract. We studied the changes in the histological structure of the gills of *Neogobius sp.* collected from the Veleka River in Bulgaria. The results showed mainly proliferative changes in the gill structure associated with proliferation of stratified epithelium in the filament and secondary lamellae. To our knowledge, this is the first histopathological study of a fish species from the river. The observed histopathological lesions could serve as useful biomarkers for monitoring water quality and, consequently, the health of species. We emphasize the importance of such research programs that monitor the fish health in rivers, considering the dynamics of various environmental factors and pollution.

Keywords: fish, gills, rivers.

Introduction

According to Fonseca *et al.* (2016), rivers present complex environments where humans and nature interact and interfere due to societal development and demographic expansion, which could cause diverse effects on freshwater quality and ecological integrity. Furthermore, according to Fonseca *et al.* (2017), fish communities are key elements for evaluating the ecological condition of rivers based on complex biomarkers. Fish histopathology is used as a biomonitoring tool for assessing aquatic quality, which gives information on the health risk of different contaminants to humans through fish consumption, with cellular alterations and damage. Furthermore, histopathological alterations in fish organs serve as biomarkers of exposure to environmental stressors. In this regard, fish gills are presented as primary target organs of environmental pollutants due to their direct contact with water and are characterized by a large surface area and absorption rates of contaminants (Pandey *et al.* 2008).

Therefore, with the present study, we aimed to evaluate the alterations in the histological structure of *Neogobius* sp. gills from the Veleka River in Bulgaria as a biomarker of freshwater quality. To the best of our knowledge, this is the first study of its kind.

Materials and Methods

The tested individuals were collected from the Veleka River, which is the largest and longest river in Strandzha Nature Park, Bulgaria. We studied *Neogobius* sp. as a bioindicator for water quality, as the Gobiidae family displays a high level of adaptability, allowing them to thrive even in the face of fluctuating environmental conditions, and their nutritional composition is similar to that of other benthophage fishes, including crustaceans, mollusks,

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worms, and insect larvae (Popov et al., 2024). Fish (n=10) were sacrificed following the humane guidelines for animals used for scientific purposes according the EU legislation. The histological method was performed according to (Romeis 1989). The assessment of the observed histopathological lesions followed Bernet *et al.* (1999).

Results and Discussion

According to Hasan *et al.* (2022) fish gill is multifunctional and responsible for important work like respiration, acid-base balance, osmoregulation, and nitrogenous excretion. Gill anatomy differs between species, but the histological structure is very similar (Wilson & Laurent 2002), and the assessment of the histological structure can serve as a biomarker in environmental assessment models. The results of the conducted histopathological study showed changes in the circulatory system of the gills, expressed in vasodilation of the main venous sinus and blood vessels in the secondary lamellae (Table 1). The observed vasodilation was a mild degree of expression. The more severe degree of change in the circulatory system, aneurysms were detected only in a single secondary lamellae and expressed in a very mild degree (Table 1). Degenerative changes affected only the stratified epithelium in the filament (Figure 1D). They were also expressed in a very mild degree. The observed proliferative changes were proliferation of the epithelium in the filament (Figure 1C), secondary lamellae (Figure 1A, B), and cells of the cartilage tissue. According to the proposed scale of Bernet *et al.* (1999), the proliferative changes were expressed in a mild degree (Table 1). The intense proliferative changes in gill epithelium could also be interpreted as defense responses of the fish, as these alterations increase the distance across which the dissolved contaminants must diffuse to reach the bloodstream (Akpakpan *et al.* 2014). The index of changes in the circulatory system ($I_{GC)}$ was determined as 6, while the index of degenerative changes ($I_{GR)}$ was determined as 3. The highest value of 12 was the index of proliferative changes (I_{GP}). The index organ was scored as Class III - moderate degree of change in the gill structure. The histopathological analysis showed a mild to moderate degree of the observed histopathological changes in the gills of the studied species. The observed lesions in the gills' histological structure were mainly proliferative, involving equally process of proliferation of epithelial cells in the filament and secondary lamellae, as well as the proliferation of chondrocytes. The alterations in the gill circulatory system were mainly concerned with vasodilation of blood vessels in the filament and secondary lamellae. Aneurysms were also found to have a higher degree of histopathological changes in the circulatory system. Similarly to our results, Fonseca *et al.* (2017) found histopathological changes such as vasodilatation, aneurysms, and degenerative changes in *Luciobarbus bocagei* and *Pseudochondrostoma* sp., and *Oncorhynchus mykiss*, collected from 6 northern Portuguese rivers. In this regard, biomarker application in environmental monitoring programmes, the evaluation and validation of biomarkers in sentinel species under different field conditions, is crucial.

In sum, our results showed a higher degree of proliferative lesions in the gills' histological structure of *Neogobius sp.* from the Veleka River. The observed histopathological lesions could be applied as useful biomarkers for water quality monitoring and thus the health of native fish species.

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Table 1. Degree of histopathological lesions in *Neogobius sp.* gills.

Reaction Pattern	Functional Unit of the Tissue	Alteration	Importance Factor (W)	Degree of the alterations - Score Value
Circulatory disturbances	*Primary Lamellae (filament)*	Vasodilation	$W_{GC1} = 1$	2
	Secondary lamellae	Vasodilation	$W_{GC2} = 1$	2
	Filament/ Secondary lamellae	Aneurysms	$W_{GC3} = 2$	1
Index for circulatory disturbances				**$I_{GC} = 6$**
Degenerative alterations	*Epithelium of primary lamellae*	Necrosis	$W_{GR1} = 3$	1
	Epithelium of secondary lamellae	Necrosis	$W_{GR2} = 3$	0
Index for degenerative alterations				**I_{GR}=3**
Proliferative alterations	*Epithelium of primary lamellae*	Edema	$W_{GP1} = 1$	0
		Proliferation of stratified epithelium	$W_{GP2} = 2$	2
		Proliferation of glandular cells	$W_{GP3} = 1$	0
		Fusion	$W_{GP4} = 3$	0
	Epithelium of secondary lamellae	Lamellar lifting	$W_{GP5} = 1$	0
		Proliferation	$W_{GP6} = 2$	2
	Cartilage tissue	Proliferation of chondrocytes	$W_{GP7} = 2$	2
Index for proliferative alterations				**I_{GP}=12**
Index organ I_G				**I_G=21**

Class I (Index ≤ 10) – normal histological structure with mild pathological changes; Class II (Index 11–20) – normal histological structure with moderate pathological changes; Class III (Index 21–30) – moderate degree of change in the histological structure; Class IV (Index 31–40) – severe degree of change in the histological structure; Class V (Index > 40) – very severe degree of change in the histological structure.

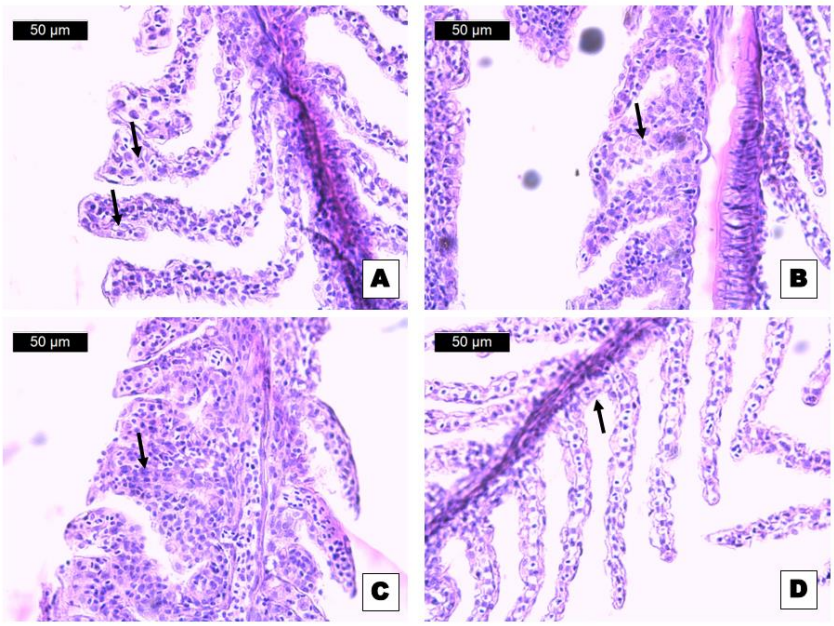

Figure 1. Gill histopathological lesions in *Neogobius* sp., staining H&E: A, B – proliferation in the secondary lamellae; C – proliferation in the primary lamellae; D – necrosis.

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