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Study of Macrophages in Common Carp (*Cyprinus Carpio*) With Special Emphasis on Gill and Swim Bladder: Biomarkers for Risk Assessment of Pollution

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ABSTRACT

Macrophage aggregates (MAs) are found in tissues of teleost fishes, including spleen, head kidney, and liver, in relation to inflammation. Many factors like age, pollution, infectious diseases are known to affect the formation, accumulation, and proliferation of macrophages in teleosts. Numerous studies have shown an increase in number and size of macrophage aggregates (MAs) in fish collected at contaminated sites. The present work has focused on morphological studies on macrophages in the head kidney, gill tissue, and swim bladder (SB) of common carp (*Cyprinus carpio*). Alive fish samples were collected from local markets, and tissues from head kidney, gills were removed. Cells were isolated. The adherent macrophages were fixed by methanol and stained by Giemsa and neutral red. The exudate from swim bladder was collected for studying macrophage cells from the inflammation. The present work showed Giemsa and neutral red stained free macrophages or macrophage aggregates on glass slides.

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The tendency of cell aggregation reflects cell to cell adhesiveness, which serving as a marker for macrophage activation. More macrophage aggregation or accumulation indicates more macrophage activation, sometimes leads to inflammation. Therefore, studies on macrophages of common carp (*Cyprinus carpio*) may be used as the markers of fish health and aquatic pollution.

Keywords: Macrophage Aggregates, Pseudopodia, Fish Health, Aquatic Pollution.

1. INTRODUCTION

Macrophage aggregates (MAs) are focal accumulations of macrophages found in the spleen, head kidney, and liver of teleost fishes. These structures are easily visualized in histologic sections. These structure contain pigments like hemosiderin, melanin, and ceroid/lipofuscin. Many factors are known to affect the formation, accumulation, and proliferation of these structures, including age [1], nutritional status [2], and infectious diseases [3]. Previous study stated the changes in various MA parameters (e.g., number, size, percent area occupied) in relation to environmental contamination and pollution [4].

The macrophages classified on basis of function into activated macrophages, wound-healing macrophages, and regulatory macrophages [5]. They also have an important role in removing cell debris and damaged components. They are also producing cytokines that participate in defensive and reparative functions [5]. Previous study [6] stated that the density of macrophages used as a marker of toxicity. A study [7] stated that the chronic inflammatory stimuli promote the immune cell aggregation. Smaller macrophages unite with each other to form the large, huge giant cells during inflammatory stimuli. Some studies [7, 8] reported the possibility of macrophage to proliferate at site of injury/ infection. Kidney tissue repair in fish is performed by interstitial cells and renal epithelial cells, supported by the macrophages [9]. The presence of macrophages at the damaged renal tubules may explain its role in the repairing process [9]. This was confirmed by other study [10] which reported that basement membrane enhances cell proliferation and differentiation through preparing growth factors.

A study [9] revealed that carp fish had relatively small macrophages. Different sizes of macrophages were identified in the study. The number and size of macrophages varied with different seasons. The activity of season may influence the size of macrophages.

Previous study [11] reported numerous macrophages as free or in aggregates (MAs) in tilapia (*Oreochromis* sp.). Formation of filopodia, attachment of charcoal particles on cell surface, cell fusion, poorly organized cell aggregation were noticed on glass slides [11]. Investigations have confirmed an increase in number and size of macrophage aggregates (MAs) and Melano-Macrophage Centres (MMCs) in tissues of fishes collected at polluted sites [11].

One study [12] reported that liver macrophage aggregates in tissue sections were poorly organized. Compared with the liver, macrophages (MACs) accumulations in the kidney tissues were larger in size and distinct. The presence of large MMCs in the livers and necrotic macrophages/ immune cell agglomerate of fishes may be related to contamination and pollution.

Researches [13] stated among head kidney leukocytes (HKL) only macrophages show the unique property of adherence on slides. The study investigated the head kidney macrophages (HKM) morphology and phagocytic behavior in teleosts [13]. Morpho-functional changes and transformations of macrophages and cell aggregates may be used as bio indicator to several environmental challenges [13].

Previous study [14] demonstrated inflammatory cells from swim bladder (SB) responding to activated charcoal particles challenge. Swim bladder (SB) of rohu (*Labeo rohita*) fishes injected with charcoal particles showed increased acute inflammation which may be important for the organism's defense against infections [14]. The present work has focused on morphological studies on macrophages in the head kidney, gill tissue, and swim bladder (SB) of common carp (*Cyprinus carpio*).

2. MATERIALS AND METHODS

2.1. Cell Isolation from Tissues

Alive fish samples of common carp (*Cyprinus carpio*) were collected (total no. 17) from local markets and dissected in lab. Tissues from head kidney, gills were removed using the forceps and mashed through the cell strainer into the petridish containing (0.1) M phosphate buffer saline (pH 7.2) in presence of trypsin- EDTA. Cell suspension was smeared directly on sterilized glass slides.

2.2. Fixation and Staining

The adherent macrophages were fixed by methanol and stained by Giemsa and neutral red. Activated charcoal particles suspended in normal saline (0.9% NaCl) was used for phagocytosis study [11,12,13]. Cell counting was done by hemocytometer.

2.3. Study of Macrophage Cells from Swim Bladder

Fishes were injected with 5ml of activated charcoal particles in normal saline (0.9% NaCl) into the swim bladder. The swim bladder was washed with saline (0.9 gm% NaCl) and trypsin, 0.09% EDTA, and the exudate was collected for studying macrophage cells from the inflammation [14].

3. RESULTS

3.1. Pseudopodia of Head Kidney Macrophages (HKM)

Head kidney macrophages (HKM) showed pseudopodia-like extensions and engulfment of activated charcoal particles.

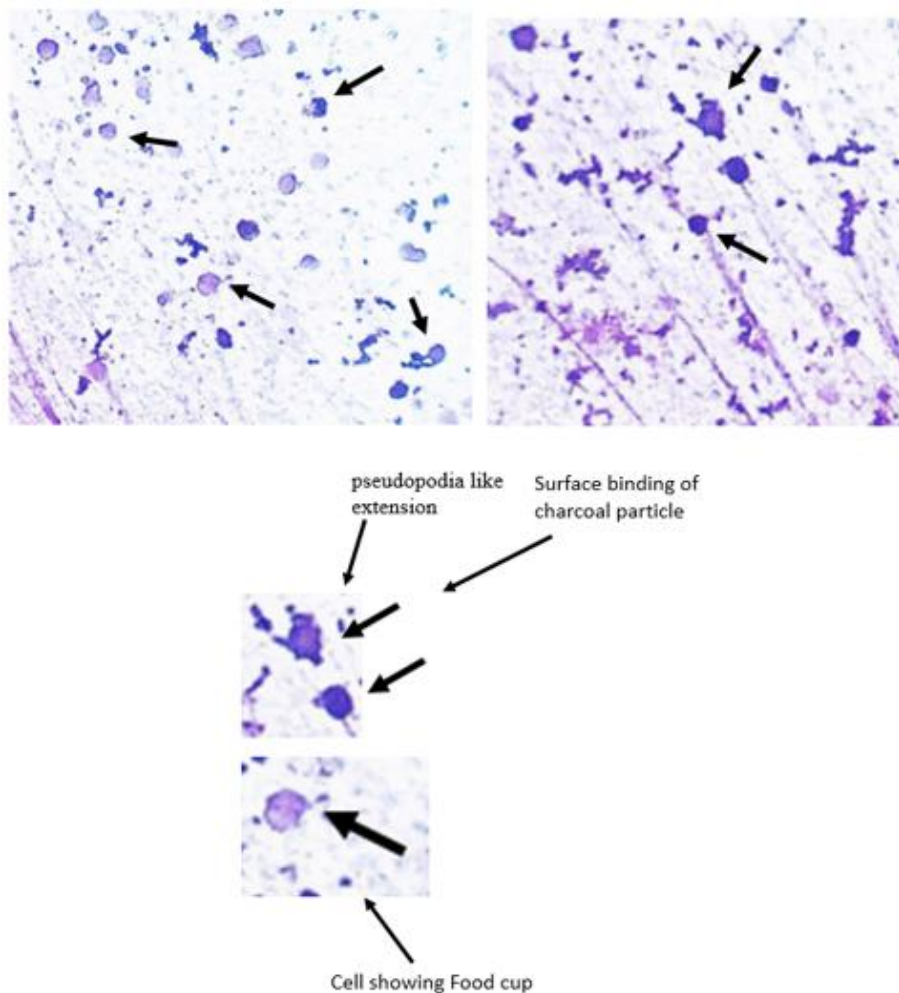


Figure1. Giemsa stained macrophages in the head kidney of common carp (*Cyprinus carpio*) on glass slides.

3.2. HKM Aggregates

Light microscopy observations showed Giemsa stained macrophages aggregates on glass slides (MAs). Lysosomal enzyme activity of macrophages was seen by neutral red in common carp (*Cyprinus carpio*).

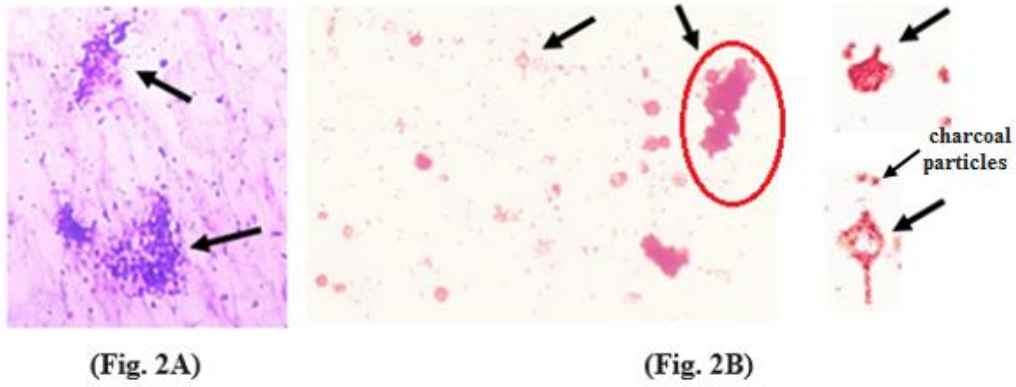


Figure 2. (A) Giemsa stained head kidney macrophage aggregates (MAs) in common carp (*Cyprinus carpio*) on glass slides. (B) Neutral red stained stages of aggregation of HKM in common carp (*Cyprinus carpio*) on glass slides challenged with activated charcoal particles.

3.3. Macrophage Aggregation of Gill Tissue

Macrophage aggregation of gill tissue in common carp (*Cyprinus carpio*) on glass slides was increased after challenge with activated charcoal particles. Neutral red positive macrophages from gill tissue were also identified.

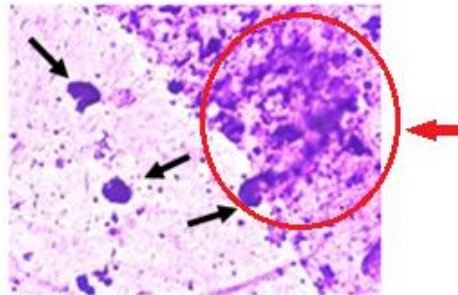


Figure 3. Free macrophages and macrophage aggregates (MAs) of gill tissue in common carp (*Cyprinus carpio*) on glass slides without challenge of activated charcoal particles.

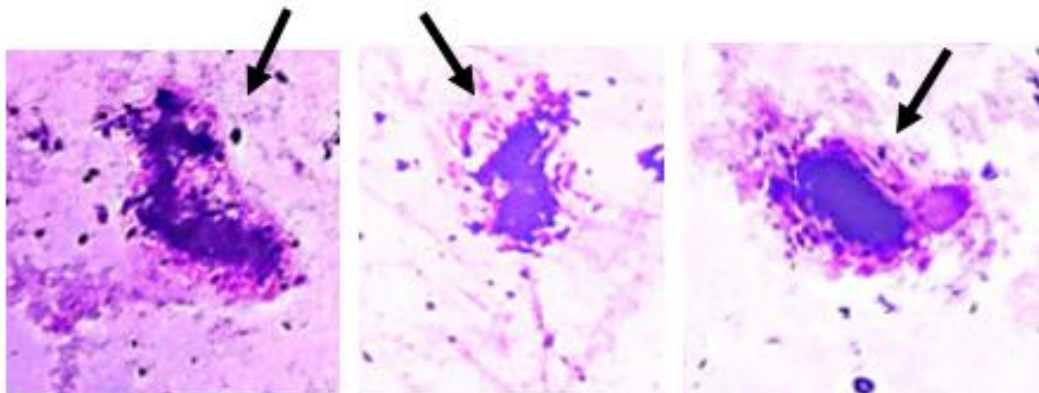


Figure 4. Macrophage aggregates (MAs) of gill tissue in common carp (*Cyprinus carpio*) on glass slides challenged with activated charcoal particles.

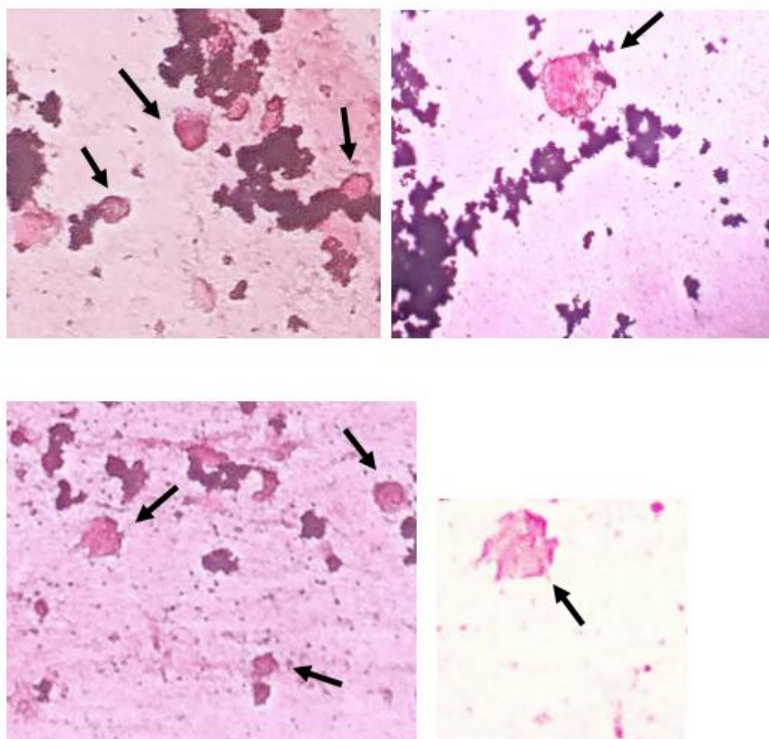


Figure 5. Neutral red positive macrophages from gill tissue in common carp (*Cyprinus carpio*) on glass slides.

3.4. Inflammatory Cells from Swim Bladder (SB)

Inflammatory cells from swim bladder (SB) responding to activated charcoal challenge was increased in number. Macrophages showed more pseudopodia like growth in the exudate collected from swim-bladder (x400) after challenging activated charcoal particles.

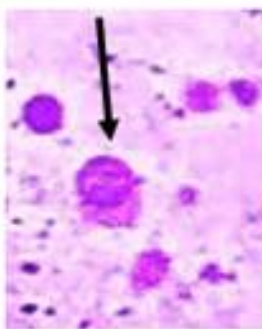


Figure 6. Inflammatory cells from SB without challenge of activated charcoal particles.

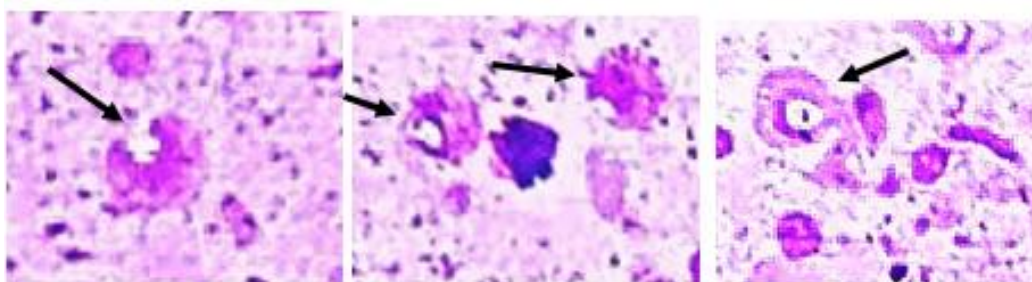


Figure 7. Inflammatory cells from SB responding to activated charcoal particles challenge.

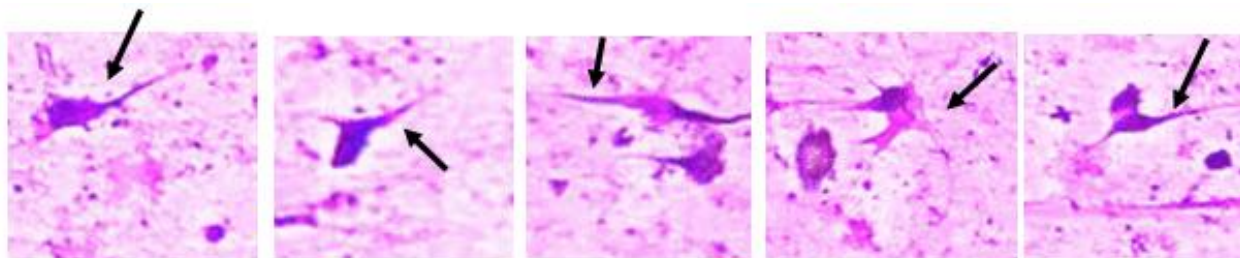


Figure 8. Macrophages showing more pseudopodia like growth in the exudate collected from swim-bladder (x400) after challenging activated charcoal particles.

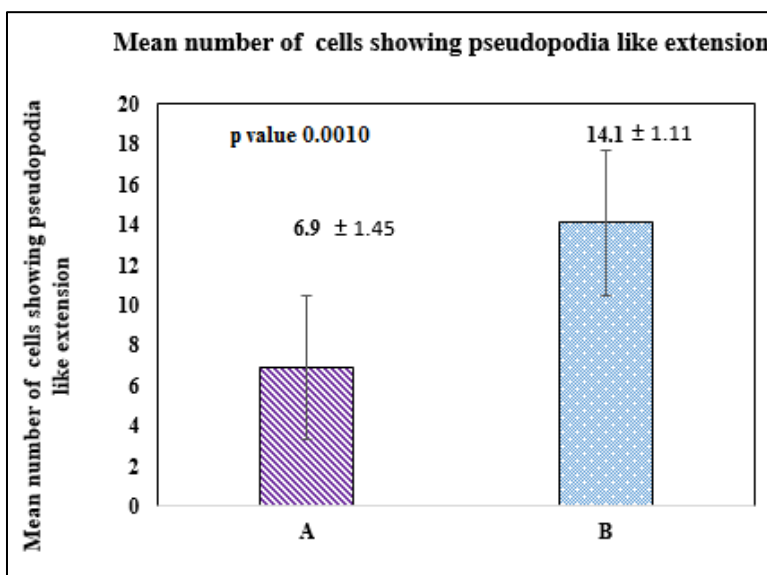


Figure 9. (A) cells without challenge of activated charcoal particles, (B) cells challenged with activated charcoal particles.

Values are expressed as Mean ± SEM. P-value < 0.05 is considered to be statistically significant.

4. DISCUSSION AND CONCLUSION

The present research has focused on macrophage biology of common carp. Because MAs are known to change in number, size in relation to environmental degradation, so they qualify as cytological biomarkers. Morpho-functional alteration of macrophages has been suggested as sensitive biomarkers of pollutant exposure and fish health [11,12,13].

Fish are in a direct contact with the waterborne pollutants [15]. Gills are the first organ to be affected by pollutants and therefore are commonly used as the markers of aquatic pollution [16]. Most studies report that various pollutants can cause alterations of the gill tissue which can be regarded as reflections of stress response [17,18]. Degenerative changes of epithelial cells of gills may be the result of the direct effects of high concentrations of xenobiotics on the gills [19]. Previous study stated [20] in order to understand the impact of gill disorders on fish health, it has to be emphasized that fish gills are essential for fish survival in the aquatic environment. The gill-associated lymphoid tissue (GIALT) of teleosts, consisting of immune cells in gill mucosa, prevents infection in gills [21]. In zebrafish (cyprinids), structures including the interbranchial lymphoid tissue (ILT) and the amphibranchial lymphoid tissue (ALT) [21] are also a site for T cell, NK cell proliferation and show structural changes after infection with spring viraemia of carp virus (SVCV), that supports their important role in immune responses of the gills [22]. A study [23] showed two distinct macrophage subsets were observed in gill epithelium: iNOS-positive macrophages, concentrated in the basal epithelium of gill suggesting a pro-inflammatory role, whereas 5-HT-positive macrophages found in the sub epithelium, contributing immune modulation. These findings revealed aquatic immune systems and environmental adaptability [23].

The present work showed Giemsa and neutral red stained free macrophages or macrophage aggregates on glass slides. The tendency of cell aggregation reflects cell to cell adhesiveness, which serving as a marker for macrophage activation [24]. More macrophage aggregation or accumulation indicates more macrophage activation, sometimes leads to inflammation.

Macrophage aggregation of gill tissue was increased after challenge with activated charcoal particles. Macrophages showed more pseudopodia like growth in the exudate collected from swim-bladder after challenging activated charcoal particles. The present work has focused on studies on macrophages of common carp (*Cyprinus carpio*) that may be used as the markers of fish health and aquatic pollution.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

- [1] Brown C L and George C T. (1985). Age-dependent accumulation of macrophage aggregates in the yellow perch *Perca flavescens* (Mitchell). *Journal of Fish Diseases*. 8, 135–138.
- [2] Agius C and Roberts R J. (1981). Effects of starvation on the melano-macrophage centres in fish. *Journal of Fish Biology*. 19, 161–169.
- [3] Vogelbein W K, Fournie J W and Overstreet R M. (1987). Sequential development and morphology of experimentally induced melano-macrophage centers in *Rivulus marmoratus*. *J Fish Biol*. 31(Suppl A), 145–153.
- [4] Wolke R E, Murchelano R A, Dickstein C D and George C J. (1985). Preliminary evaluation of the use of macrophage aggregates (MA) as fish health monitors. *Bulletin of Environmental Contamination and Toxicology*. 35, 222–227.
- [5] David M M and Justin P E. (2008). Exploring the full spectrum of macrophage activation. *Nature Reviews Immunology*. 8(12), 958-969.
- [6] Couillard C M, Hodson P V. (1996). Pigmented macrophage aggregates: A toxic response in fish exposed to bleached-Kraft mill effluent. *Environmental Toxicology and chemistry*. 15(10), 1844-1854.
- [7] Minton K. (2016). Granuloma macrophage differentiation. *Nature Reviews Immunology*. 16, 718-719.
- [8] Robbins CS, Hilgendorf I and Weber G F. (2013). Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat. Med*. 19(9), 1166-1172.
- [9] Amer M Hussin and Suhaib AHA Al-Taai. (2018). *Journal of Entomology and Zoology Studies*. 6(2), 24-27.
- [10] Mesher A L. (2010). *Junqueira's Basic Histology*. 12th ed. McGraw Hill Medical. 222.
- [11] Guria S. (2024). Study Of Macrophages from Spleen Of Tilapia (*Oreochromis* Sp.) To Evaluate Immune Mechanisms In Fish. *IJBPAS*. 13(3), 1422-1429
- [12] Guria S and Ali R. (2024). A mechanistic insight into the cytomorphology of liver macrophages in tilapia (*Oreochromis* sp.). *Indian J. Applied & Pure Bio*. 39(2), 665-670.
- [13] Guria S. (2023). A comprehensive approach to health assessment of fish population using head kidney derived macrophages (HKM) and macrophage aggregates (MA) from rohu (*Labeo rohita*) and tilapia (*Oreochromis* sp.): the potentially sensitive biomarkers of contaminant exposure and several environmental stressors. *Indian J. Applied & Pure Bio*. 38(3), 1502-1509.
- [14] Guria S, Middya O, Hossain A B and Saha D. (2023). Assessment of Inflammatory Cells in The Exudate Collected From Swim Bladder Of Rohu (*Labeo rohita*). *Bull. Env. Pharmacol. Life Sci*. 12 (9), 48-53.
- [15] Roberts R J. (1989). *Fish pathology*. 2nd edition. Baillière Tindall, London.

- [16] Bernet D, Schmidt H, Meier W, Burkhardt-Holm P and Wahli T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases*. 22, 25-34.
- [17] Heath A.G. (1995). *Water pollution and fish physiology*. Lewis publishers, Boca Ration.
- [18] Ferguson H W, editor. (2006). *Systemic Pathology of Fish: A Text and Atlas of Normal Tissues in Teleosts and their Responses in Disease*. 2nd edition. Scotian press, London.
- [19] Koca Y B, Koca S, Yildiz S, Gürcü B, Osañç E, Tunçbas O and Aksoy G. (2005). Investigation of histopathological and cytogenetic effects on *Lepomis gibbosus* (Pisces: Perciformes) in the Çine stream (Aydin/Turkey) with determination of water pollution. *Environmental Toxicology*. 20, 560-571.
- [20] Zawisza M, Chadzinska M, Steinhagen D, Rakus K, Adamek M. (2024). Gill disorders in fish: Lessons from poxvirus infections. *Rev Aquac*. 16(1), 234-253. doi:10.1111/raq.12835
- [21] Rességuier J, Dalum AS, Pasquier LD, Zhang Y, Koppang EO, Boudinot P, Wiegertjes GF. (2020). Lymphoid tissue in teleost gills: variations on a theme. *Biology*. 9(6), 127. doi: 10.3390/biology9060127.
- [22] Dalum AS, Kraus A, Khan S, Davydova E, Rigaudeau D, Bjørgen H, López-Porras A, Griffiths G, Wiegertjes GF, Koppang EO, Salinas I, Boudinot P, Rességuier J. (2021). High-resolution, 3D imaging of the zebrafish gill-associated lymphoid tissue (GIALT) reveals a novel lymphoid structure, the amphibranchial lymphoid tissue. *Front Immunol*. 12, 769901. doi:10.3389/fimmu.2021.769901
- [23] Hussein M T, Zacccone G, Albano M, Alesci A, Marino S, Alonaizan R, and Mokhtar D M. (2025). Serotonin Signaling and Macrophage Subsets in Goldfish Gills: Unraveling the Neuroimmune Network for Gill Homeostasis. *Life*, 15(5), 751. <https://doi.org/10.3390/life15050751>
- [24] Hodgkinson J W, Grayfer L and Belosevic M. (2015). Biology of Bony Fish Macrophages. *Biology*. 4(4), 881-906. <https://doi.org/10.3390/biology4040881>