



## Histological and histochemical characterization of gill tissue in common carp (*Cyprinus carpio*)

Yahia Dahash Hamdi<sup>1</sup>; Diyar Mohammad Hussein<sup>2</sup>;  
Ali A. Tala'a<sup>3</sup>; Rasha M. A. Baldawi<sup>4\*</sup>

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### Abstract

**Background:** Teleost fish possess gills that are very complex and multifunctional structures. The histopathological and histochemical integrity of teleost gills is an excellent indicator of overall fish physiological health status and the health of the aquatic environment. **Objective:** The study aimed to characterize the microstructure of gill tissue and the distribution of mucopolysaccharides in healthy *Cyprinus carpio* gills using routine and specialized staining methods as a baseline. **Methodology:** A total of fifteen adult male common carp (approximately 280 g each and approximately four months average age) were collected from commercial aquaculture farms located along the Euphrates River in Anbar Province, Iraq. Gill arches were removed from each specimen, immersed in 10% neutral buffered formalin and subsequently processed by routine procedures. The histologic evaluation of gill sections stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), and Alcian blue (pH 2.5) under the light microscope demonstrated normal gill architecture (primary and secondary lamellae) and normal cell morphology (pavement, chloride, pillar, mucus, chondrocyte, and erythrocytes) to be found in all fifteen specimens examined (100% concordance across all specimens). **Results:** Mucous cells in the gill sections demonstrated strong magenta PAS staining in all specimens, which indicates a large amount of neutral mucopolysaccharides with consistent staining intensity of 3+ (strong). Alcian blue staining of the gill epithelial chloride cells and gill cartilage demonstrated the presence of the acidic mucopolysaccharides, which showed moderate-to-strong staining intensity of 2+ to 3+. The absence (0% incidence) of pathological changes (lamellar fusion, epithelial lifting, or necrosis) in any of the (n=15) specimens further validates the findings contained in this study as a healthy reference standard for subsequent studies concerning *Cyprinus carpio* gills regarding toxicity testing, environmental monitoring, and comparative evaluations of aquatic species health.

1- Department of Anatomy and Histology, College of Veterinary Medicine, University of Fallujah, Al Anbar, Iraq. Email: dr.yahia\_hamdi@uofallujah.edu.iq, ORCID: <https://orcid.org/0000-0003-3223-6694>

2- Department of Anatomy and Histology, College of Veterinary Medicine, University of Al-Muthanna, Iraq. Email: dmh201094@mu.edu.iq, ORCID: <https://orcid.org/0000-0002-4515-4180>

3- Department of Anatomy and Histology, College of Veterinary Medicine, University of Fallujah, Al Anbar, Iraq. Email: alialrawi@uofallujah.edu.iq, ORCID: <https://orcid.org/0000-0002-1035-4051>

4\*- Middle East University College, Baghdad, Iraq. Email: rasha.mohammed@meuc.edu.iq, ORCID: <https://orcid.org/0009-0005-9611-1059>

\*Corresponding author

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Conclusion: the current study's findings were suggestive of a predominantly neutral mucin phenotype in healthy common carp gills from aquaculture sources during non-challenged conditions.

**Keywords:** *Cyprinus carpio*, Gill histology, Histochemistry, PAS staining, Alcian blue, Mucopolysaccharides, Baseline characterization

## Introduction

The common carp has become a mainstay in aquaculture today and accounts for a large share of freshwater fish production worldwide. It is considered one of the more significant fish species produced via aquaculture in many inland areas of Asia, Europe, and the Middle East. The ability of common carp to adapt to so many different environments is why it is one of the few fish species found in abundance in both natural water systems and commercial fisheries worldwide. Additionally, common carp is one of the freshwater fish species farmed along the Euphrates and Tigris rivers in Iraq and is a very important source of animal protein for the populations in these areas (Al-Helli, Resen and Ali, 2019; FAO, 2022).

Gills are among the most complex and functionally significant structures in a teleost's anatomy. Each gill is made up of a cartilaginous arch that is connected by multiple (or primary) filaments, also referred to as holobranchs. Each primary filament has multiple secondary lamellate structures that arise perpendicular to it and provide a large surface area for gas exchange (Evans, Piermarini and Choe, 2005; Olson, 2002). The gill (mucosal) epithelium is made up of several distinct cell groups: pavement cells (which are squamous epithelial cells that are the first line of protection and barrier), chloride cells (ionocytes that are responsible for active transport of ions and

osmoregulation), pillar cells (myoepithelial cells that structurally support blood channels through the lamellae of the gills), and mucous producing cells (goblet cells that produce protective mucins). Collectively, all of these functionally distinct cells of the gill epithelium facilitate respiration and gas exchange through the exchange of anions ( $\text{CO}_2$ ) and cations ( $\text{O}_2$ ); also aid in the regulation of ions, acid–base balance, osmotic homeostasis, and nitrogenous waste through this exchange (Evans, Piermarini and Choe, 2005). Due to its close physical contact with the surrounding aqueous environment, the gill epithelium is exceptionally sensitive to fluctuations in water quality and to contamination by chemical substances (Strzyzewska, Szarek and Babinska, 2016). There is considerable evidence that reproduction of histopathological lesions in the gills of fish can occur as a result of exposure to heavy metals, pesticides, pharmaceutical contaminants, and alterations of physical/chemical parameter values (Jabeen *et al.*, 2018; Carvalho *et al.*, 2020; Wu *et al.*, 2022), with examples of gill lesions including lamellar fusion, lifting of the epithelium, hyperplasia of chloride cells, hypertrophy of mucous cells, and necrosis of gill tissue. As a result, gill histopathology is a widely utilized bioindicator of the ecological and toxicological condition of aquatic ecosystems (Yancheva *et al.*, 2016; Marinović *et al.*, 2021).

Evaluating the gill epithelium histochemically enhances an evaluation of functional ability in the fish via the biochemical types and distribution of mucopolysaccharides, which can be done through both PAS (Periodic Acid–Schiff) staining and Alcian blue staining. Staining (PAS) is used for neutral mucopolysaccharides and glycoproteins, which are major constituents of the protective mucous membrane that covers the gill surface (Pereira and Caetano, 2009; Smith *et al.*, 2018). Conversely, at a pH of 2.5, Alcian blue stains acidic mucopolysaccharides like glycosaminoglycans with sulphate or carboxyl groups; these mucopolysaccharides are significant structural constituents of the gill arches (cartilage matrix), and have variable amounts in chloride cells and other epithelial cell types (Danguy 2009; Carmona *et al.*, 2004). Therefore, the variances in the histochemical profile of both neutral and acidic mucopolysaccharides show both structural traits and functional nature of the gill tissue.

To this date there are few studies documenting histopathologic alterations of fish gills related to environmental stress and pesticide exposure (Jasim *et al.* 2022, Marinović *et al.* 2021, Fu *et al.* 2021), but there are no studies documenting the histological and histochemical characteristics of healthy gills of *Cyprinus carpio* from the Euphrates River Basin in Iraq. To accurately assess tissue-level alterations in experimental and comparative environmental studies, a morphologically valid and accurately documented reference from a well-established

baseline of normality must be referenced that is specific to the population and geographic area being studied. There is considerable methodological difficulty in distinguishing pathological changes from normal physiological and/or adaptive responses because there is no consensus as to establishing the normal baseline for the population and geographic area being studied.

This study will provide a baseline reference of healthy gill tissue from common carp, *Cyprinus carpio*, therefore establishing a foundation for future ecotoxicological and health assessments of aquatic environments in Iraq. The objectives of this study include: (i) establishing the normal histological organization of gill tissue from juvenile common carp obtained from commercial aquaculture operations in the Euphrates River; (ii) determining the distribution of neutral and acidic mucopolysaccharides using both PAS and Alcian blue staining; and (iii) to develop a permanent long-term reproducible reference for healthy gill tissue of *Cyprinus carpio* for ecotoxicological assessments of aquatic environments in Iraq.

The structure of this study is as follows: summaries from the literature on teleost gill histology and histochemistry, and the use of gills to monitor environmental ecosystem health is presented in Section 2; materials and methods are provided in Section 3; results of histological and histochemical analysis of the gill tissue of common carp are presented in Section 4; and the results of the present study are compared to previously published studies and any limitations presented in Section 5.

The final section contains a summary and recommendations for future research.

## Literature Review

### *Structural Organization of Teleost Gills*

Teleost gills have been extensively described as structurally organized, and are fairly conserved. In general, the teleosts are usually provided with four pairs of gill arches, supporting primary filaments (holobranchs), which give rise to a multitude of secondary lamellae extending perpendicularly, thus increasing the area of the surface of gills exchange (Danguy 2009; Wegner, 2023). This hierarchical structure of diffusion of respiratory gases is efficient and is of great importance in osmoregulation and metabolic exchange.

The vascular system of gills is an intensive arrangement of the lamellar blood stream upheld by pillar cells, which allow the blood and the external water to be close to each other (Olson, 2002). The general structural arrangement is maintained though there are quantitative changes that may occur with change of species and environmental factors like surface area of lamellar, epithelial thickness, and interlamellar space (Wegener, 2011).

There are a number of specialized cells in Gill epithelium, which perform respiratory, osmoregulatory and protective functions, such as pavement cells, chloride cells (ionocytes), pillar cells and mucous cells (Roberts, 2003). Comparative research has shown that although these cellular component parts are specific, dense, and uniform, they can differ depending on certain ecological adaptations and feeding mechanisms (Alsafy *et al.*, 2025).

Gill tissues are further characterized histochemically to contribute to the functions. The most common method of identifying neutral mucopolysaccharides and glycoproteins found in mucous cells is periodic acid-Schiff (PAS) staining, and acidic glycosaminoglycans are identified with the help of Alcian blue (pH 2.5) used in cartilage and epithelial structures (Carmona *et al.*, 2004; Smith *et al.*, 2018). The joint AB/PAS is used to give a detailed information about mucin structure and localization in gill tissues (Suvarna, Layton and Bancroft, 2019).

Gill histopathology has also been identified as a sensitive biomarker of environmental stress as well as aquatic pollution. The exposure to the effects of heavy metals, pesticides, and organic pollutants has been linked to the typical lesions: the lamellar fusion, the lifting of the epithelial cell, the changes in mucous cells, and the necrosis (Carvalho *et al.*, 2020; Wu *et al.*, 2022). The regional literature, especially the studies of Iraqi river systems, has noted that it is crucial to set baseline histological references to determine the changes caused by the pollution (Jasim *et al.*, 2022; Al-Helli, Resen and Ali, 2019).

There is still the absence of region-specific baseline data available on histological and histochemical features of *Cyprinus carpio* gills in the Euphrates River Basin due to the availability of vast literature. This base information is necessary in differentiating normal physiological states with pathological changes in the environmental and toxicological research.

## Materials and Methods

### *Animals and Sample Collection*

This represented fifteen (15) adult male common carp produced in three (3) commercial aquaculture farms in the Euphrates River in Anbar Province, Iraq in July 2022. The fish were four (4) months in age, and of average weight 280 grams (245 to 320 grams) and total length of 23.4 centimeters on average (some were 21.0 cm. long, others 25.0 cm. long). The fish were sampled considering they had no disease, external parasites and/or injuries (macroscopically). Pre-sampling (aeration) of the fish was done to reduce the impact of handling stress and all specimens were euthanized following accepted veterinary procedures, and experimental use of fish in this study was authorized by the relevant ethical committee and in compliance with the institutional policies regarding the use of experimental animals in a humane manner.

After euthanasia via cervical transection, the operculum was removed from each fish to expose the underlying gill arches. The gill arch and gill tissue were then rinsed with phosphate-buffered saline (0.9% NaCl, pH 7.2) to wash away any blood and surface mucus, and any adherent adipose and connective tissue were carefully trimmed from the gill tissue using 10X magnification. All specimens were dissected and placed into fixative (10 minutes – 15 minutes postmortem) in an attempt to minimize any postmortem autolytic changes to the gill tissue.

### *Tissue Fixation and Processing*

The gill arches that were taken out from the fish were fixed using 10% neutral

buffered formalin for approximately 24-48 hours at room temperature. The fixative-to-tissue ratio was 10:1; therefore, it was important to maintain adequate fixative to ensure complete penetration. After being fixed, the gill arches were cut into sections (3-5mm thick) to allow adequate penetration of the processing reagents. The sections were then dehydrated in increasing ethanol concentrations (70%, 80%, 95%, 100%) for 60 minutes each at room temperature. The sections were then “cleared” by placing them in two changes of xylene (30 minutes each) following the dehydration step. The sections were then infiltrated with paraffin wax by placing them in two changes of molten paraffin (melting point 56-58°C) in a vacuum oven at 60°C, each for 60 minutes, prior to being embedded in paraffin blocks using an embedding station. Sections of 5-6micron thickness were cut on a calibrated rotary microtome (Leica RM2125, Germany) and collected on glass microscope slides coated with albumen, and allowed to air dry overnight in a 37°C oven. All tissue processing was performed using accepted histological procedures described in (Suvarna, Layton and Bancroft, 2019).

### *Staining Procedures*

The three staining protocols performed on all fifteen specimens included:

- i. Hematoxylin and Eosin (H&E): The serial sections first underwent deparaffinization in xylene, and were rehydrated through a 100%, 95%, and 70% ethanol series before going to distilled water. The sections were then stained with Mayer’s haematoxylin for 8 minutes, followed by differentiation in 1%

- acid alcohol (5 seconds), bluing in running tap water (5 minutes), and counterstaining with eosin Y solution (0.5% aqueous) for 3 minutes. All sections were then dehydrated, cleared, and mounted using DPX.
- ii. Periodic Acid-Schiff (PAS): After deparaffinizing and hydrating the serial sections, were oxidized using 1% aqueous periodic acid for 10 minutes and were rinsed in distilled water, subsequently treated with Schiff's reagent for 15 minutes in the dark at room temperature, followed by a wash in running tap water for 10 minutes, counterstained with Mayer's haematoxylin for 2 minutes and dehydrated before mounting.
  - iii. Alcian Blue (pH 2.5): The serial sections were deparaffinized and hydrated before being treated with Alcian Blue solution (1% Alcian Blue 8GX in 3% acetic acid, pH 2.5) at room temperature for 30 minutes, rinsed in distilled water, counterstained for 5 minutes with nuclear fast red before being dehydrated, cleared in xylene, and mounted.

In all cases, the procedures followed the standard methods of (Suvarna, Layton and Bancroft, 2019) and incorporated positive tissue controls for all staining runs.

#### *Microscopic Examination and Documentation*

Each section of tissue was then analysed using an Olympus BX53 binocular light microscope (Japan) with a calibrated Olympus DP73 digital camera at 40x-400x magnification, using four

objective lenses (4x, 10x, 20x, 40x). A total of 135 stained sections were analysed (n=15 animals x 3 stains x 3 sections). Staining intensity in mucous cells was scored semi-quantitatively on a four-point scale (0 = negative, 1+ = weak, 2+ = moderate, 3+ = strong) according to the histochemical morphological criteria established by (Danguy, 2009), Smith *et al.* (2018). High-resolution JPEG Photomicrographs (minimum of 300 dpi) were obtained using the cellSens standard imaging software provided with Olympus and will be submitted to the journal as separate files. Each figure legend will include a scale bar and a description of the stain used.

#### *Statistical Considerations*

Although formal analysis of inferential statistics was not the main purpose of the study due to its descriptive and observational character, a semi-quantitative assessment of staining intensity scores for all specimens and all sections collected from each specimen was made to allow for a quantitative assessment of staining characteristics in the sample population using mean  $\pm$  standard deviation of scores. To evaluate the extent to which cellular identification and staining results for each section and animal were consistent, a descriptive analysis of data from individual and all specimens was used to determine the overall concordance.

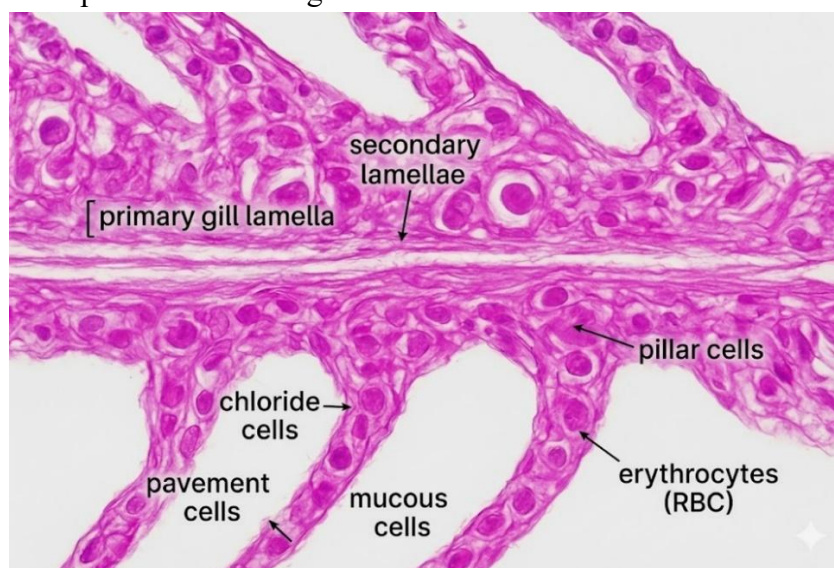
## **Results**

### *General Histological Architecture (H&E Staining)*

Staining of all fifteen specimens using hematoxylin and eosin, which showed 100% Agreement, revealed clearly distinct gill architecture with primary

filaments extending from the gill arch cartilage to support bilaterally arranged secondary lamellae (Figure 1). All sections exhibited a clear delineation of the basement membrane, which is a distinct layer of extracellular material that separates the epithelium covering the

gill from the underlying connective tissue. A stratified epithelium surrounds the primary filaments, and the structure is made up of layers of connective tissue containing collagen fibres, fibroblasts, and blood vessels.



**Figure 1: Histological section of common carp (*Cyprinus carpio*) gill showing the primary lamella (PL), secondary lamellae (SL), pavement cells (PVC), chloride cells (CC), pillar cells (PC), mucous cells (MC), and chondrocytes (Ch). Scale bar = 50  $\mu$ m, (H&E,  $\times 40$ ).**

Lining the surfaces of the lamellae were the pavement cells whose flattened morphology appeared eosinophilic in cytoplasm and dark, oval-shaped basophilic nuclei. The chloride cell populations (ionocytes) were primarily located at the base of secondary lamellae and closest to the primary filaments, exhibiting granular, eosinophilic cytoplasm and centrally located, large basophilic nuclei. The pillar cells form the structural component of the blood channels of the lamellae, which are characterised by their flanged cytoplasmic processes and basophilic nuclei. Mucous cells are present in both single and small clusters in the epithelium. Identifiable by their expanded, light-staining cytoplasm due to the mucous secretory granules within

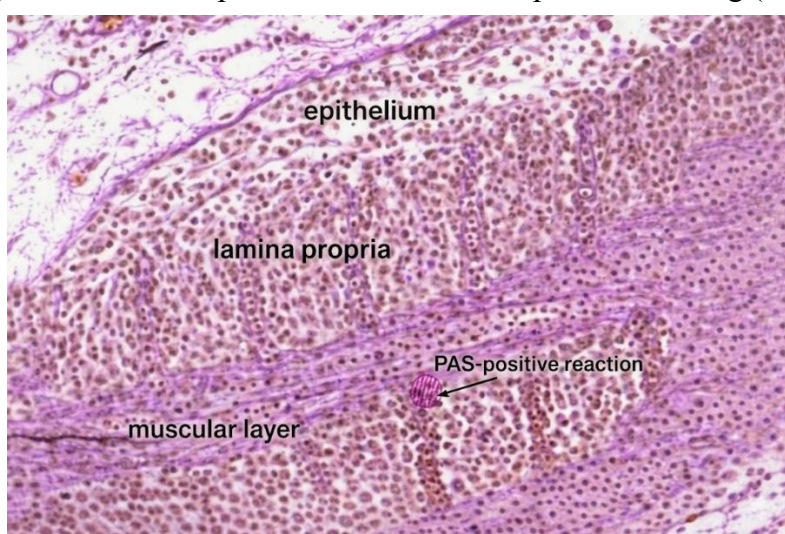
them. Chondrocytes found within the cartilaginous cores of the gill arches and primary filaments displayed basophilic nuclei situated in lacunae surrounded by a lightly stained cartilaginous matrix. Erythrocytes in the capillaries of the lamellae exhibited uniformly eosinophilic cytoplasm and centrally located nuclei; these results support the characteristic structure of teleost erythrocytes as nucleated. None of the fifteen specimens exhibited any pathological alterations, including lamellar fusion, epithelial lifting, aneurysms, or necrosis.

#### *PAS Staining – Neutral Mucopolysaccharides*

PAS showed that the mucous cells found in almost all sections consistently exhibited a strong magenta stain

( $2.9 \pm 0.2$  mean, 2-3+ range), indicating a high level of neutral mucins and glycoproteins in their secretory granules (Figure 2). Mucous cells were present throughout the primary and secondary lamellar epithelial cells, but were more densely packed in the primary filaments than in the secondary lamella. There was a wide range of PAS-positive staining results (1+-2+) in the surface epithelium

of pavement cells, due to their glycocalyx, which contains glycoproteins. The cartilage matrix of the gill arches exhibited moderate positive staining, which would suggest that chondroitin is the main component of the cartilage matrix due to it containing a high level of glycoproteins. Chloride and pillar epithelial cell types showed low to no PAS-positive staining (0-1+).

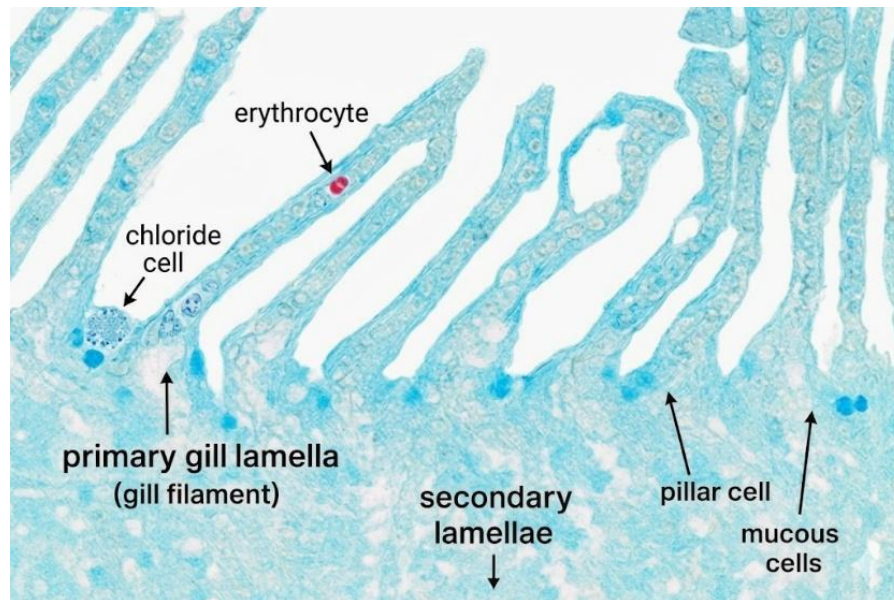


**Figure 2: Histological section of common carp gill demonstrating strong magenta reaction in mucous cells (MC, arrowheads), moderate PAS positivity is noted in the cartilaginous matrix (CM) and surface epithelium. scale bar = 50  $\mu$ m, PAS ( $\times 40$ ).**

#### *Alcian Blue Staining – Acidic Mucopolysaccharides*

At a pH of 2.5 using Alcian blue, there were variable levels of acidic mucopolysaccharide in gill cell populations (Figure 3). Chloride cells had blue cytoplasm (score of 2+ to 3+) and red-stained nuclei using nuclear fast red counter stain, indicating that there are acid glycosaminoglycans present in moderate levels in the cytoplasm. The chondrocytes of the gill arch cartilage exhibited strong positive staining with Alcian blue (score of 3+), with purple

cytoplasm and red nuclei, indicating that the cartilaginous matrix contains high levels of sulphated glycosaminoglycans. Pavement and pillar cells had low, variable levels of positive Alcian blue staining (score of 0 to 1+). Mucous cells showed very weak Alcian blue staining (score 0 to 1+), indicating that the mucins in this group are primarily neutral rather than acidic, based on the healthy baseline conditions studied. Therefore, in healthy unstressed carp, gill mucous cells are primarily composed of neutral mucin-secreting (PAS-positive) goblet cells.



**Figure 3: Histological section of common carp gill illustrating acidic mucopolysaccharides. chloride cells (CC) show moderate-to-strong blue cytoplasmic staining, while chondrocytes (CH) exhibit strong alcian blue positivity. mucous cells (MC) demonstrate weak reactivity, scale bar = 200  $\mu$ m, alcian blue stain ( $\times 10$ ).**

### Discussion

Based on both histologic and histochemical analyses, the present study provides a cohort-specific and geographically contextualized baseline characterization of gill tissue microstructure and mucopolysaccharide composition in healthy common carp (*Cyprinus carpio*) raised on aquaculture farms along the Euphrates River in Anbar Province, Iraq. The results using H&E, PAS, and Alcian blue staining found in this study are mostly consistent with prior descriptions of normal teleost gill histology (Danguy 2009; Smith *et al.*, 2018; Alsafy *et al.*, 2025), adding a novel reference point for the Iraqi aquatic research community in terms of population-specific and geographically contextualized data.

The H&E staining profile of specimens examined in this study, showing granular eosinophilic cytoplasm and dark basophilic nuclei within pavement, chloride, pillar, and mucous

cells, was entirely consistent with basic principles of staining histology and previously published descriptions of normal teleost gill cytoarchitecture (Smith *et al.*, 2018). The clear delineation of the basement membrane, well-organized lamellar arrangement, and absence of pathological changes in all 15 specimens examined support the conclusion that all specimens were in a healthy physiological state. The above observations agree with those reported by (Alsafy *et al.*, 2025), who confirmed that structurally intact gill tissue in freshwater teleosts consistently exhibits clearly delineated epithelial layers, well-vascularized secondary lamellae, and distinct cellular boundaries. Additionally, the purple H&E-stained chondrocyte cytoplasm supports the view that the basophilia of cartilage matrix components is attributable to the presence of highly sulfated glycosaminoglycans, which provide negative charge (Roberts, 2003). The strong, consistent PAS staining of gill

mucous cells (mean  $\pm$  standard error, SE;  $2.9 \pm 0.2$ ) is consistent with previous morphological and histochemical characterizations of these cells in teleost gills from healthy, unstressed animals (Smith *et al.*, 2018; Wegner, 2011). The inner mucous barrier is comprised of neutral mucins and glycoproteins that are generated by gill goblet cells and serve as a vital defence of physicochemical barrier to pathogens, abrasive, and irritants dissolved in surrounding water (Pereira and Caetano, 2009). The high PAS reactivity shows that the gill mucous cells were in an active secretory condition during the period of assessment. In contrast, the previously observed decrease in PAS+ gill mucous cells in experimentally stressed cyprinids reflects a toxic insult to these structures.

The significant loss of AB-PAS+ gill mucous cells in gibel carp (*Carassius gibelio*) after delta-methrin exposure, as illustrated by (Wu *et al.*, 2022), was attributed to the exhaustion of mucous cells and/or immunotoxicity. The high PAS positive staining in the present study may provide a quantitative reference for future studies of comparative toxicology, as there is no evidence of gill mucosal damage and/or chemical insult in the present data. Other than the Alcian blue reaction in gill cells observed in the present study showed moderate to high levels of staining in gill chloride cells (2+ to 3+ stained cells) and high levels of staining in gill chondrocytes (3+ stained cells), with very low levels of staining in gill mucous cells (0 to 1+ stained cells). Under normal conditions in *Cyprinus carpio*, the present results suggest that gill mucous cells are primarily producers of neutral mucin. That chloride cells are

likely the predominant source of acidic mucopolysaccharides within the gill and the cartilaginous matrices. The histochemical profile of gill mucous cells in teleosts without any external stressors has been well established by (Danguy, 2009; Malhotra *et al.*, 2020). A dynamic response of gill mucous cell histochemistry to environmental stressors is well-documented, (Marinović *et al.* 2021) recorded a large range of variability in proportion and chemical characteristics of mucous cells over seasonal water quality and levels of environmental stressors. Therefore, the data obtained in the current work can be used as a valuable indicator of the objective assessment of the changes in the composition of mucopolysaccharides under the influence of environmental stress.

In no specimen observe evidence of pathology; that is, lamellar fusions, lifting of epithelium, aneurysms, clubbing of second lamellae, mucous cell hyperplasia, or necrosis. The lesions are reported in teleosts (such as carp) that are exposed to heavy metals, pesticides, and organic pollutants (Jabeen *et al.*, 2018; Fu *et al.*, 2021; Wu *et al.*, 2022) and are commonly identified as signs of environmental stress. Thus, the absence of such lesions in this study can be taken as evidence that studied were living under water quality conditions acceptable to them, as indicated by their being free from significant exposure to toxins at the time of specimen collection, and, as such, constitute healthy baseline reference specimens.

### *Limitations of the Study*

As a matter of methodological transparency, recognize several limitations of this study. The first limitation is that the study did not include adult male and female common carp in this gill study because only male common carp were sampled during the single seasonal sampling period in July 2022. This may limit this ability to determine male and female gill histochemistry, the age of cells that make up the gills of male and female adult carp, and to determine gill histochemistry based on gill histological changes that occur seasonally; therefore, the results may not accurately represent the histology and histochemistry of the gills of both sexes of adult common carp.

Secondly, the study sampled only three aquaculture farms in one geographic location along the Euphrates River in Anbar Province. Therefore, the baseline data may not represent all carp populations from river systems or environmental conditions in Iraq. Finally, although the study used a semi-quantitative scoring of staining intensity (e.g., score of 0–3), did not include any stereological or morphometric quantification (e.g., mucous cell density by length of epithelium or surface density of chloride cells); therefore, the quantitative comparison of the study's results to data from other studies may be less precise.

Furthermore, the study did not include scanning or transmission electron microscopy to quantify cell surface features, the number and characteristics of cell tight junctions, or the number and types of cell organelles; therefore, additional validation of the histological

findings was not available. Lastly, at the time of sample collection, several water quality parameters in both holding and farm waters were not measured; therefore, it cannot be excluded that minor environmental changes between aquaculture farms could have been confounding factors. Future research should address the limitations of this study by using multiple-season sampling and multiple-site sampling designs, with morphometric evaluation and the collection of water quality data.

### **Conclusions**

The current study describes the first comprehensive histological and histochemical characterization of the gills of healthy adult common carp (*Cyprinus carpio*) from commercial aquaculture farms along the Euphrates River Basin in Iraq, and it has been systematically corroborated. The principal findings of the current study can be summarized as follows: (i) All fifteen specimens exhibited the presence of normal (non-pathological) histological alterations with respect to their lamellar structure (e.g., primary and secondary overall lamellar structure) and that all structures (pavement cells, chloride cells, pillar cells, mucous cells, chondrocytes, and erythrocytes) were clearly defined in all specimens, and (ii) overall, mucous cells exhibited neutral mucopolysaccharides (mean score of  $2.9 \pm 0.2$ ) as demonstrated by a strong reactivity to PAS staining, thereby indicating the functional integrity of the mucosal gill barrier, and (iii) while the mucous cells were weakly reactive to Alcian blue staining, the chloride cell and cartilage storage tissues demonstrated moderate to strong acidic

mucopolysaccharides; therefore, the current study's findings were suggestive of a predominantly neutral mucin phenotype in healthy common carp gills from aquaculture sources during non-challenged conditions.

The significance of the current study's findings, as compared to those of previous studies, is threefold. First, the present study provides a geographically and population-specific historical and histochemical baseline for the gills of *Cyprinus carpio* in the Euphrates River Basin. Second, the quantitative data generated from staining intensity scores provide objective, quantifiable baselines against which future studies of histochemical alterations due to toxic exposure, pollution, and environmental monitoring will be conducted. Finally, the present study documented that no pathological changes in the gills were observed in farm-raised common carp; consequently, the histological methods applied in the current study have been further validated and have supported the use of farm-raised common carp as an appropriate reference animal for comparative studies.

#### Declarations

**Conflict of Interest:** The authors declare no conflicts of interest.

**Funding:** This research received no specific external funding.

**Ethics Statement:** All animal procedures were conducted in accordance with institutional ethical guidelines for the humane use of experimental animals.

**Data Availability:** Raw data are available from the corresponding author upon reasonable request.

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