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Seasonal Histomorphological Study of the Gills of Common Carp (*Cyprinus carpio*) in the Tigris River, Iraq

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Abstract **- The present study aims to describe the morphological and histological characteristics of the gills in male Common Carp (Cyprinus carpio) across four seasons and to highlight the seasonal effects on gill structures under varying temperature conditions. For this purpose, 52 specimens were collected from a rearing aquarium in the Tigris River, within the Taji region of Baghdad, over the course of a year. The fish were sacrificed, and the specimens were fixed in a 10% neutral buffered formalin solution. Routine histological techniques were applied, and the samples were stained using Hematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS), and Van Gieson (VG) stains. Morphological analysis revealed that the gills of C. carpio consisted of four pairs of gill arches located inside the branchial chamber, equipped with gill rakers and secondary lamellae, all covered by the operculum. The epithelium of the primary lamellae comprised several cell types, including chloride cells (ionocytes), which were rarely observed in the secondary lamellae, as well as pavement cells, non-differentiated supporting cells, and primary epithelial cells. The secondary lamellar epithelium contained all these cell types except for non-differentiated cells and additionally featured pillar cells. Histologically, the gill raker epithelium was identified as a non-keratinized stratified squamous type containing goblet (mucous) cells and two types of taste buds: elongated superficial and spherical. The gill arches' blood supply was provided by afferent branchial arteries (ABAs) and efferent branchial arteries (EBAs), while afferent filament arteries (AFAs) and efferent filament arteries (EFAs) supplied the primary lamellae. The secondary lamellae were vascularized by afferent and efferent lamellar arterioles. The results demonstrated significant differences ($P < 0.05$ and $P < 0.01$) in gill epithelium characteristics across the four seasons. Statistical analyses revealed that inter-lamellar distance varied significantly ($P < 0.01$) between seasons, which was attributed to temperature fluctuations. The study concluded that carp could adapt to a limited temperature range (23–30°C). However, when water temperatures dropped below the lower threshold (8°C) during winter, the carp's ability to adapt diminished, leading to increased histological changes compared to other seasons.**

Keywords: **Gill morphology, Histological Analysis, Common Carp (Cyprinus carpio), Seasonal Variations, Temperature adaptation**

I. INTRODUCTION

The Common Carp (*Cyprinus carpio*) is a freshwater species found in habitats such as large rivers, lakes, and standing ponds across Asia and Europe. Common carp are widespread globally and are of ecological concern due to their vulnerability to extinction; however, they are often regarded as a destructive invasive species [1]. Native to Asia and Eastern Europe, common carp thrive in standing or slow-moving waters with soft, vegetative sediments [2]. Common carp belong to the family *Cyprinidae*, one of the largest groups of freshwater fish in Asia and Eastern Europe. Globally, more than forty thousand species of fish inhabit seas, oceans, lakes, and rivers, with common carp contributing significantly to the diversity of freshwater species [3]-[5]. They are vertebrates primarily found in seas, ponds, large rivers, and lakes [6]. Common carp prefer shallow waters and inhabit the middle and lower parts of rivers, favoring temperature ranges between 23°C and 30°C.

They can survive low winter temperatures but cannot tolerate water salinity levels exceeding 5%. Common carp thrive in oxygen concentrations between 0.3-0.5 mg/L and can adapt to supersaturated environments [7]. Their diet includes benthic organisms such as insect larvae, aquatic insects, worms, mollusks, and zooplankton. They also forage in turbid waters by digging at the bottom [8]. The average weight of carp ranges between 0.6-1.0 kg body weight (b.w.) in one season (three months) and can reach up to 1.5 kg b.w. after three seasons (nine months) [9]. Carp species are categorized into wild carp, which have poorly understood genetic structures, and farmed carp, which are extensively studied [10]. Fish lack lungs, relying instead on gills for respiration. Gills facilitate gas exchange between blood channels in the secondary lamellae and water carrying dissolved oxygen. The gill epithelium functions as a filter and is structurally similar to the branchial basket of protovertebrates. The epithelial cells of gills are joined by tight junctions of varying depth and express diverse transport functions [3], [11].

II. OBJECTIVES OF THE STUDY

1. To describe the morphological and histological features of gills and kidneys during four seasons.
2. To analyze the impact of seasonal stress on gills and kidneys under varying temperature conditions.

A. Materials and Methods

The study included 52 healthy male Common Carp (*Cyprinus carpio*), approximately two months old. Fish samples were collected from a single aquarium during each

season of the Tigris River by commercial fish breeders in the Taji region of Baghdad city (Fig. 1). The fish were captured during four seasons: autumn, winter, spring, and summer. In the autumn season, specimens were caught in October, while in the winter season, specimens were collected in January. During the spring and summer seasons, specimens were captured in March and August, respectively. The weather conditions in Baghdad city during the collection months (October, January, March, and August) recorded temperatures ranging between 11°C and 42°C throughout the year and across all seasons. Meanwhile, the water temperature in the aquarium environment during specimen collection ranged between 8°C and 31°C across the four seasons.

Fig. 1 Map of Illustrating Study Through the Specimens Collection of Tigris River of Taji Region in Baghdad City During Four Seasons (Google Earth)

Thirteen specimens from each season were used. The average length of the fish ranged from (21.31±0.29 cm) to (32.61±0.88 cm), and the average weight ranged from (296.61±22.83 g) to (521.92±20.79 g). Morphological measurements of the gills, including length and weight, were conducted for analysis. The gills were first washed with normal saline, and adipose tissue was carefully removed. The dissected specimens were prepared within a period of 15-20 minutes.

Dimensional measurements of the morphology were taken using a ruler to measure the length (in cm) from the anterior to the posterior extremity. The weight of the gills (in g) was recorded using a sensitive electronic balance. Volume measurements of the gills were performed within 15-25 minutes after dissection. These measurements were obtained using a volumetric cylinder filled with water. The sample was immersed in the water, and the difference in water levels before and after immersion was recorded. After measurements, the gills were fixed in 10% buffered formalin solution.

The formalin-to-sample volume ratio was maintained at a 10:1 ratio to ensure proper fixation. Each sample was labeled and placed in a container with formalin. Regular checks were conducted to ensure all parts of the samples were fully immersed in the formalin solution and not adhering to the container's sides or bottom. In some cases, glass wool was added to the container to prevent the samples from sticking. For histological preparations, the gills were washed thoroughly with tap water for 5-10 minutes and then fixed in 10% buffered formalin.

B. Methods

The water temperature was recorded using a thermometer during the months of specimen collection in each season throughout the year. Weather conditions were documented using the official weather reports for Baghdad city.

C. Measurements and Morphometric Analysis

The average surface area of the respiratory lamellae and the general length of the primary lamellae were evaluated after removal from the fixative. Thirteen gill samples from *C. carpio* were analyzed. The fifth primary lamellae from each hemibranch located on the left side of the second gill arch of each carp were measured. Measurements included the length of the primary lamellae and the secondary lamellae. For the secondary lamellae, measurements were taken at the base, middle, and tip (Fig. 2). The gill arch was divided into five regions, with each region measuring 0.5 cm in unit length.

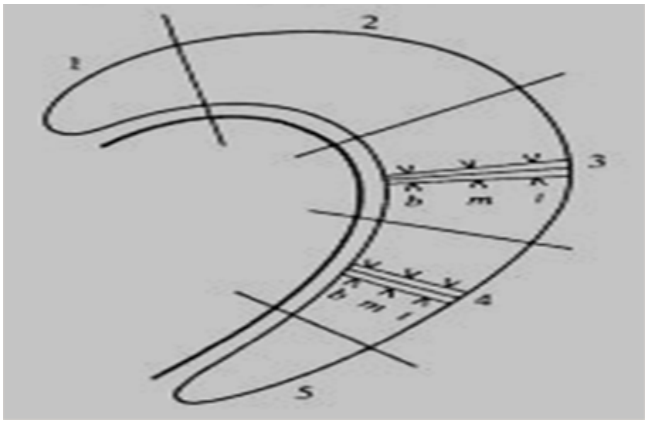

Fig. 2 Explain Hemibranch of the Gills Divided into Five Regions and the Secondary Lamellae Divided into Base, Middle and Tip on the Filament [12]

III. RESULTS AND DISCUSION

A. Normal Morphology of Fish Gills

The present study revealed that the gills of *C. carpio* are situated on both sides of the pharynx, within a cavity called the branchial chamber (Fig. 3). This study examined a closed branchial system with a single external branchial aperture (Fig. 4). The branchial aperture, a bony structure with a flap-like shape, functions to cover and protect the internal organs (gills) and is referred to as the operculum (Fig. 3). Generally, the gills consist of three main parts: the rakers, the gill arch, and the filaments (also called primary lamellae). Additionally, the secondary lamellae are located on either side of the filaments (Fig. 5). The primary lamellae (filaments) are elongated extensions that arise from the gill arch. The gill arch is a cartilaginous structure that supports the primary lamellae and has a V-shaped configuration, commonly referred to as holobranchs. Each holobranch consists of paired halves called hemibranches. Furthermore, each holobranch carries two hemibranches, a finding consistent with the results reported by [13][3][14][15].

These studies also revealed that teleost fish (bony fish) have a bony operculum as an external feature that covers and protects the gills. The gills consist of rakers, gill arches, primary lamellae, and secondary lamellae. The nature of teleost gills is classified as holobranchs, meaning complete gills, which include a gill arch supported by cartilage or bone. This structure supports the primary lamellae (filaments) and hemibranches (half of a complete gill). Each holobranch in bony fish carries a pair of hemibranches.

Fig. 3 Photograph Showed that Cyprinus Carpio and its Operculum (Red Arrow) During Four Seasons

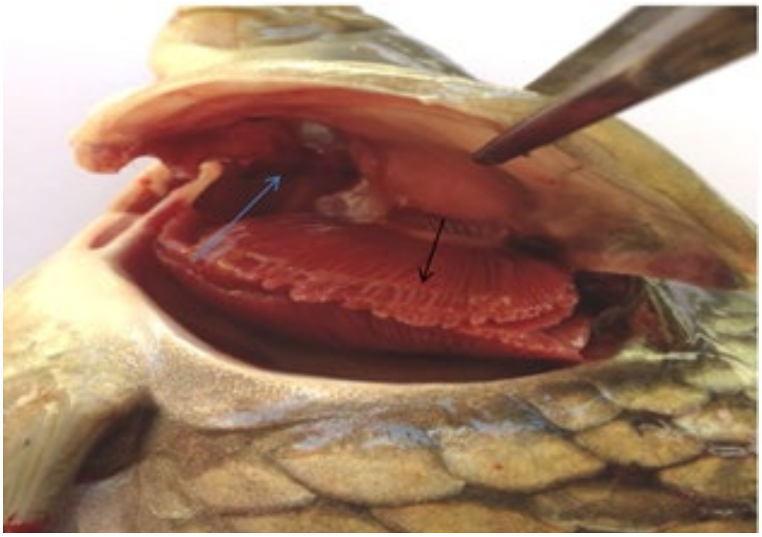

Fig. 4 Photograph Showed that Single External Branchial Aperture of Branchial Chamber (Blue Arrow) and the Gills Arch Lie Inside it (Black Arrow) During Four Seasons

B. Gill Rakers

The study revealed that the gill rakers were composed of two rows on each gill arch. These rakers were located on the anterio-medial ridge of the gill arch and were characterized by their short length, pointed structure, prominent appearance, stiffness, firmness, and tooth-like shape (Fig. 5). The gill rakers were primarily non-respiratory in function. Their main role was to act as a sieve-like structure for filtering water from food particles that entered along with the water during gill movement.

This mechanism prevented foreign food particles from passing into the gill passages. Additionally, the gill rakers served to protect the delicate primary lamellae (filaments) from hard food particles. This filtration process removed larger food particles before they entered the digestive tract via the esophagus. These findings are consistent with the results reported in [15][16][17], which stated that gill rakers are short, thick processes originating from the internal surface of the gill arch. These studies also noted the irregular spacing between rakers in fishes, with wider spacing observed in species that feed on larger prey, depending on the type of diet. Furthermore, gill rakers were described as facilitating the filtration of aggregated substances, forming a sieve to prevent damage to the fragile primary lamellae during water flow into the gill passages. However, the present study disagreed with [15], which reported that gill rakers form a single row on the gill arch.

C. Gill Arch and Primary Lamellae

The study found that *C. carpio* possesses four pairs of gill arches located on both sides of the fish, covered by a bony structure called the operculum on each side (Figs. 3 & 4). The gill arches are supported by cartilaginous and skeletal structures, including hyaline cartilage and adipose tissues composed of loose connective tissue.

The primary function of the gill arches is to support the gill primary lamellae, providing a framework for the gill filaments (primary lamellae) (Fig. 5). These findings align with the results of several previous studies [16][17][3][18], which demonstrated that bony (teleost) fish have four pairs of gill arches.

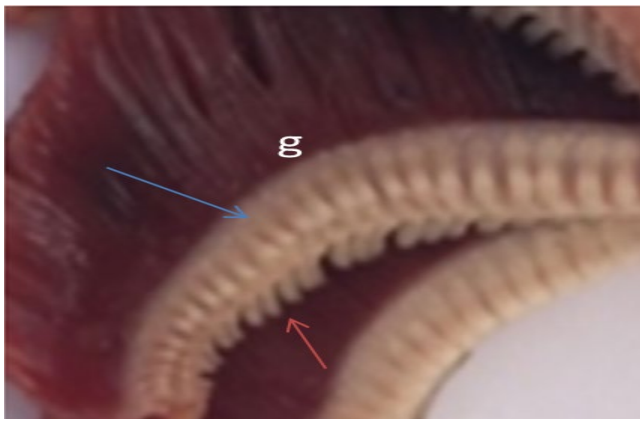

Fig. 5 Anatomical Photograph of Four Paired Gill's Hemibranches During Four Seasons Showed that A: Gills Rakers (Red Arrow), Gill Arch (Blue Arrow) and Filaments (g)

D. Normal Histology of Fish Gills

Each gill raker consisted of loose connective tissue, including adipose tissue, smooth muscle fibers, and collagen fibers of hyaline cartilage, which provide structural support to the gill raker. Gill rakers appeared as finger-like prominences extending from the gill arch, with irregular spaces between them, known as inter-raker spaces. The distances between rakers varied depending on the fish species. The primary function of gill rakers was to filter water from food particles entering the aquatic medium (Figs. 6 & 7).

Gill rakers contained aggregations of mucous (goblet) cells (Figs. 8 & 9). The study observed that the epithelial cells covering the surfaces of primary and secondary lamellae were separated from the underlying connective tissue by an extracellular material layer termed the basement membrane, which also supported the gill raker epithelium (Figs. 8 & 9). Additionally, another type of taste bud with a spherical shape was identified. These intraepithelial taste buds in the gill rakers contained chief cells with centrally located sensory cells and satellite cells located peripherally (Fig. 11). Mucous cells aided in capturing or arresting small food particles, preventing them from passing toward the esophagus. In addition to mucous cells, taste buds extended from the basement membrane through the gill rakers (Fig. 9). These elongated taste buds functioned as chemical receptors, assisting in the selection of food particles for swallowing. Taste buds were categorized into two types: elongated taste buds, located near the epithelial surface (superficial), and characterized by dark-colored elongated nuclei surrounded by pale-colored supporting cells, and spherical taste buds. Both types contained two kinds of cells: supporting cells and neuroepithelial cells, which were derived from the stem cells of the taste buds (Fig. 8).

The epithelium lining the gill raker was identified as non-keratinized stratified squamous epithelium (Figs. 8 & 9). The study, using a light microscope, showed that the gills of *C. carpio* were covered by epithelium in both the primary (non-respiratory) and secondary (respiratory) lamellae, which exhibited a distinctive barrier between the fish's aquatic environment and its cellular fluids.

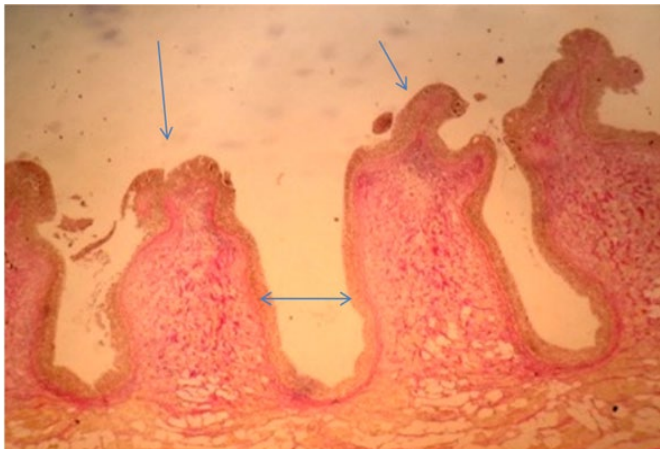

Fig. 6 Longitudinal Section of Gill's Hemibranch During Four Seasons Showed that Gill Rakers (Blue Arrow) and Inter Rakers Space (Transverse Arrow), X40 VG Stain

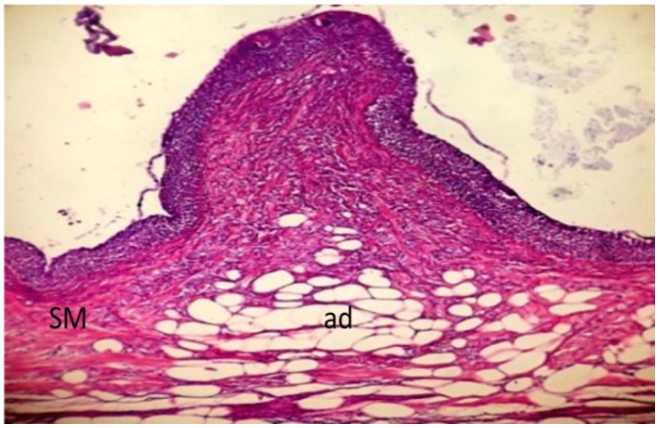

Fig. 7 Longitudinal Section of Gill's Raker During Four Seasons Showed that Adipose Tissue (adp) and Smooth Muscle Fibers (Sm), X100 H&E Stain

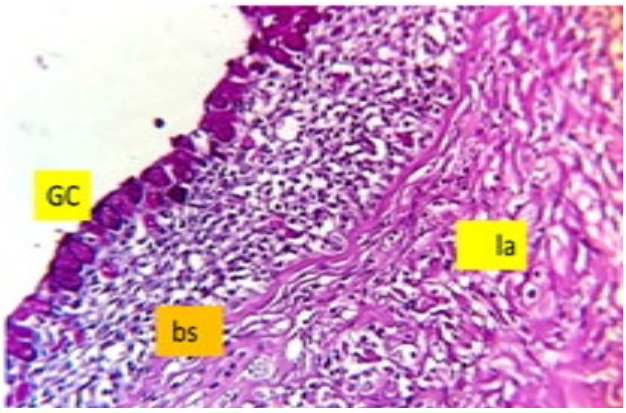

Fig. 8 Sagittal Section of Gill's Raker During Four Seasons Showed that A: Mucous (Goblet) Cells (GC) and Thickening of Basement Membrane (Bas) B: Mucous (Goblet) Cells (GC), X400 PAS Stain

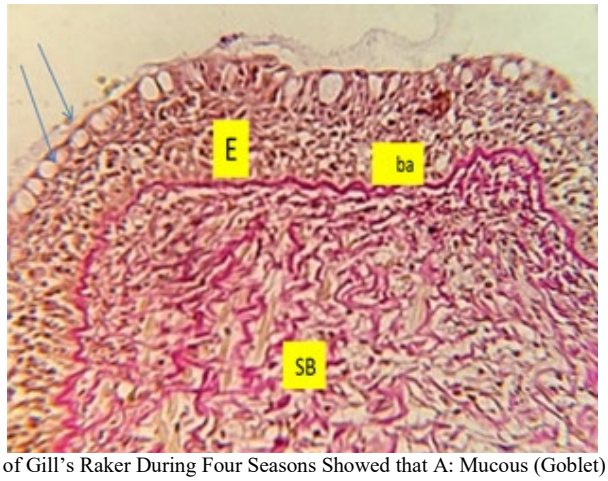

Fig. 9 Cross Section of Gill's Raker During Four Seasons Showed that A: Mucous (Goblet) Cells (Blue Arrow), Submucosa (Sm), Basement Membrane (Bas) and Non- Keratinized Stratified Squamous Epithelium (Epi). X400 VG Stain

The findings are consistent with those of [16], [19], and [20]. Numerous blood vessels occupy the regions of the gill arch, surrounded by afferent and efferent branchial arteries (Fig. 10). The primary lamellae, on the other hand, are supported by hyaline cartilage composed of chondrocytes. Afferent blood vessels transport deoxygenated blood to the secondary lamellae, while efferent blood vessels carry oxygenated blood away from the secondary (respiratory) lamellae. These vessels contribute to anastomosing blood vessels that form the central venous sinus, which is connected to the efferent filament arteries (Fig. 11). These observations are in agreement with [3], [16], [20], and [13].

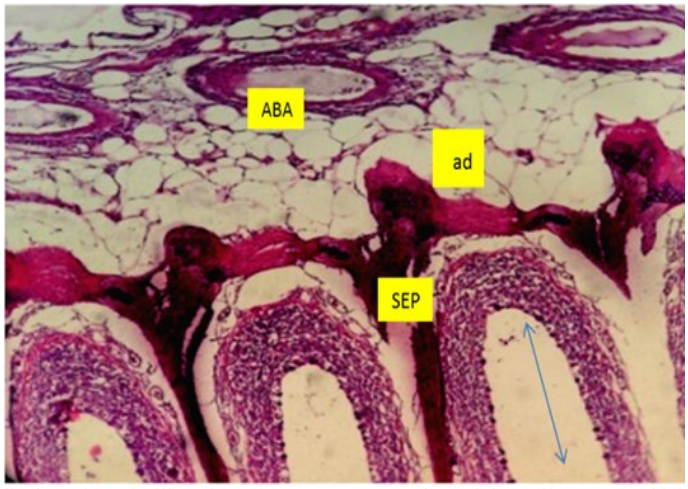

Fig. 10 Longitudinal Section of Gill Arch During Four Seasons Showed that Afferent Branchial Arteries (Abas), Adipose Tissue (Adp), Inter Branchial Septum (Sep) and Filaments (Fil), X100 H&E Stain

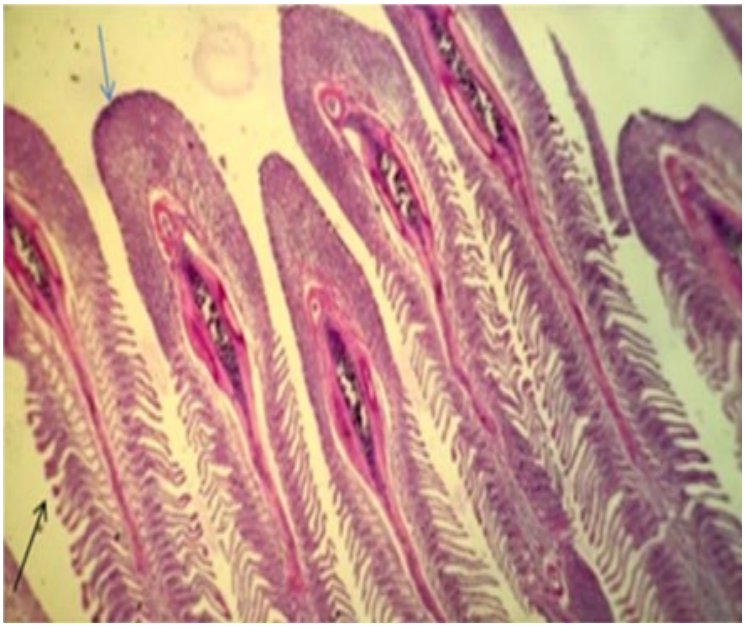

Fig. 11 Longitudinal Section of Gill During Spring Season Showed that A: Bulging of Tip of Filaments (Blue Arrow) and Lamellae (Black Arrow), X40 H&E Stain

TABLE I EXPLAINED THE VALUES OF TEMPERATURE LEVELS OF WATER AND WEATHER DURING FOUR SEASONS AND THEIR MONTHS IN BAGHDAD CITY

Seasons	Months	Water Temperature(C°)	Weather Temperature(C°)
Autumn	October	24 C°	29 C°
Winter	January	8 C°	11 C°
Spring	March	18 C°	22 C°
Summer	August	31 C°	42 C°

TABLE II ANATOMICAL DIMENSIONS OF CARP'S BODY AND GILLS DURING FOUR SEASONS

Seasons	Fish length (cm)	Fish Weight (g)	Fish gill length (cm)	Fish gill weight (g)	Fish gill volume (cm^3)
Autumn	29.54±0.98 A	444.62±30.81 A	4.80±0.10 A	4.07±0.10 A	5.05±0.04 A
Winter	21.31±0.29 B	296.61±22.83 B	2.94±0.06 B	2.09±0.11 B	2.74±0.06 B
Spring	27.43±0.19 C	403.84±25.26 A	4.44±0.09 C	3.49±0.07 C	4.01±0.04 C
Summer	32.61±0.88 D	521.92±20.79 C	5.14±0.08 D	4.75±0.08 A	5.17±0.06 A
(P<)	0.01	0.01	0.01	0.01	0.01

TABLE III NUMBERS OF THE GILL'S FILAMENTS AND LAMELLAE AND DIMENSIONS OF CARP'S GILL'S RESPIRATORY SURFACE AREA DURING FOUR SEASONS

Seasons	Gill Filament Number per 0.5 cm	Gill Lamellae Number per Filament	Filament Length (µm)	Lamellae Length (µm)	Lamellae Width (µm)	Gill's Barrier (Thickness of Lamellae Epithelial Cells with its Space) (µm)	Blood Channel Width or Blood Lucuna (µm)	Gills Inter Lamellar Space or Distance (µm)
Autumn	10.00±0 A	122.85±0.17 A	5079.6±194.6 A	133.40±0.63 A	48.57±0.11 A	4.67±0.03 A	6.72±0.03 A	17.89±0.01 A
Winter	10.00±0 A	123.15±0.19 A	4225.8±135.4 B	87.25±0.17 B	45.44±0.11 B	3.65±0.02 B	5.20±0.03 B	16.75±0.01 B
Spring	10.00±0 A	122.90±0.20 A	4979.6±227.1 A	101.97±0.15 C	50.90±0.09 C	4.97±0.03 A	7.71±0.04 C	17.14±0.02 C
Summer	10.00±0 A	123.05±0.20 A	5186.1±169.41 A	133.85±0.45 A	49.21±0.06 D	6.81±0.06 C	7.80±0.01 C	18.87±0.02 D
(P<)	NS	NS	0.01	0.01	0.01	0.01	0.01	0.01

IV. CONCLUSION

The study concluded that temperature fluctuations directly impact gill tissue in the aquatic environment across the four seasons. Although carp can adapt within a temperature range of 23–30°C, the recorded water temperatures were 8°C in winter and 31°C in summer, exceeding the species' adaptation limits. Consequently, significant cellular injury (histological changes) was observed in this study.

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