Revista de Biología Tropical, ISSN: 2215-2075, Vol. 73: e63718, enero-diciembre 2025 (Publicado Jul. 29, 2025)

VERTEBRATE BIOLOGY

REVISTA DE Biología Tropical

https://doi.org/10.15517/rev.biol.trop..v73i1.63718

# Morphology, histology and histochemistry of the gastrointestinal tract of the fish *Prochilodus magdalenae* (Characiformes: Prochilodontidae)

Andrés Felipe Monsalve-Blandón[1]; https://orcid.org/0009-0005-7791-6089
Irina López-Montaño[1]; https://orcid.org/0009-0000-1250-8635
Ana Estrada-Posada[2]; https://orcid.org/0000-0003-3585-3719
Mariana Gutiérrez-Espinosa[3]; https://orcid.org/0000-0001-6127-9955
Gersson Vásquez-Machado[4]; https://orcid.org/0000-0002-4737-7038
Jonny Yepes-Blandón[1]*; https://orcid.org/0000-0001-6276-5488

1. Grupo de Investigación en Organismos Acuáticos Nativos y Exóticos (GIOANE), Universidad de Antioquia, Medellín, Antioquia, Colombia; afelipe.monsalve@udea.edu.co, irina.lopez@udea.edu.co, jonny.yepes@udea.edu.co (Correspondencia*)
2. ISAGEN S.A. E.S.P, Medellín, Antioquia, Colombia; aestrada@isagen.com.co
3. Centro de Estudios e Investigación en Acuicultura CEIAC, Guamal, Meta, Colombia; marianacgutierreze@gmail.com
4. HISTOLAB, Bogotá D.C., Cundinamarca, Colombia; gmvasquezm@unal.edu.co

Received 30-I-2025. Corrected 21-IV-2025. Accepted 24-VI-2025.

## ABSTRACT

**Introduction:** The Bocachico *Prochilodus magdalenae*, an endemic herbivorous/detritivorous fish from the Magdalena River basin, is the region's most important fishing resource, with high ecological and socioeconomic significance. Its populations have declined significantly due to fishing pressure and anthropogenic environmental stressors, including wastewater discharge, infrastructure projects, and material extraction. Understanding the characteristics of its digestive tract is a crucial knowledge gap, hindering the comprehension of nutritional and physiological processes vital for aquaculture and both *in situ* and *ex situ* conservation efforts.

**Objective:** To characterize the morphological, histological, and histochemical features of *P. magdalenae's* gastrointestinal tract (GIT) and relate them to its feeding habits.

**Methods:** Fifteen adult specimens from the Sogamoso River (Santander, Colombia) were analyzed using standard morphological measurements and histological and histochemical staining techniques for tissue structure and mucin distribution analysis. Descriptive statistics were used for morphological analysis.

**Results:** Specimens averaged 24.66 cm standard length, 291.77 g weight, and 1.02 condition factor, with a mean GIT weight of 12.02 g. The digestive tract showed four distinct regions: oropharyngeal cavity, esophagus (mean length 1.26 cm), stomach, and intestine (mean length 88.18 cm). The esophagus contained abundant acinar glands, producing neutral and acidic mucins, with acidic mucins predominating. The U-shaped stomach showed simple columnar epithelium with tubular gastric glands producing only neutral mucins. The intestine, lined with simple columnar epithelium, exhibited differential mucin distribution: high neutral mucins anteriorly and predominantly acidic mucins in middle and posterior segments.

**Conclusions:** The gastrointestinal characteristics align with its feeding habits, particularly the elongated intestine and thick pyloric muscular layer. The distinct mucin distribution pattern, especially the carboxylated acidic mucins in the middle and posterior intestinal segments, supports nutrient absorption functions. These findings provide crucial reference points for fish welfare assessment, pathogen response in aquaculture, and environmental impact evaluation in natural habitats.

**Key words:** histochemistry; histology; mucins; mucous cells; detritivorous fish.

## RESUMEN

## Morfología, histología e histoquímica del tracto gastrointestinal del pez *Prochilodus magdalenae* (Characiformes: Prochilodontidae)

**Introducción:** El Bocachico *Prochilodus magdalenae*, un pez herbívoro/detritívoro endémico de la cuenca del río Magdalena, es el recurso pesquero más importante de la región, con alta importancia ecológica y socioeconómica. Sus poblaciones han disminuido significativamente debido a la presión pesquera y los estresores ambientales antropogénicos, incluyendo vertimientos, proyectos de infraestructura y extracción de materiales. Comprender las características de su tracto digestivo es un vacío crucial de conocimiento, que dificulta el entendimiento de los procesos nutricionales y fisiológicos vitales para la acuicultura y la conservación *in situ* y *ex situ*.
**Objetivo:** Caracterizar los aspectos morfológicos, histológicos e histoquímicos del tracto gastrointestinal (TGI) de *P. magdalenae* y relacionarlos con sus hábitos alimenticios.
**Métodos:** Se analizaron quince especímenes adultos del río Sogamoso (Santander, Colombia) mediante mediciones morfológicas estándar y técnicas de tinción histológicas e histoquímicas para el análisis de estructura tisular y distribución de mucinas. Se empleó estadística descriptiva para el análisis morfológico.
**Resultados:** Los especímenes promediaron 24.66 cm de longitud estándar, 291.77 g de peso y 1.02 de factor de condición, con un peso medio del TGI de 12.02 g. El tracto digestivo mostró cuatro regiones distintas: cavidad orofaríngea, esófago (longitud media 1.26 cm), estómago e intestino (longitud media 88.18 cm). El esófago contenía abundantes glándulas acinares que producen mucinas neutras y ácidas, con predominio de las ácidas. El estómago en "U" mostró epitelio columnar simple con glándulas gástricas tubulares con solo mucinas neutras. El intestino, revestido de epitelio columnar simple, exhibió distribución diferencial de mucinas: mucinas neutras en la parte anterior y mucinas ácidas en los segmentos medio y posterior.
**Conclusiones:** Las características gastrointestinales se alinean con los hábitos alimenticios, particularmente el intestino elongado y la gruesa capa muscular pilórica. El patrón de distribución distintivo de mucinas, especialmente las mucinas ácidas carboxiladas en segmentos intestinales medios y posteriores, sustenta funciones de absorción de nutrientes. Estos hallazgos proporcionan puntos de referencia cruciales para la evaluación del bienestar de los peces, la respuesta a patógenos en acuicultura y la evaluación del impacto ambiental en hábitats naturales.

**Palabras clave:** histoquímica; histología; mucinas; células mucosas; peces detritívoros.

## INTRODUCTION

The Bocachico (*Prochilodus magdalenae*), an endemic herbivorous/detritivorous fish from the Magdalena River basin, is the region's most important fishing resource, with high ecological and socioeconomic significance. In Colombia, total fishing of *P. magdalenae* has significantly decreased, dropping from 38 000 tons in 1978 to 6 000 tons in 1999 (Mojica et al., 2012), representing an 84 % decline. By 2018, this quantity reduced to 47.9 % of the total capture (Doria-Gonzalez et al., 2020), raising concerns among environmental authorities regarding the utilization of this hydrological resource due to its vulnerable conservation status in Colombian territory (Mojica et al., 2012). This species holds substantial socio-economic importance and contributes to ecological equilibrium and nutrient mobilization (Albus, 2012; Allan & Castillo, 2007; Couto et al., 2021; Flecker, 1996). Is also important to note that fish represent the most diverse and abundant vertebrates globally, encompassing approximately 34 000 known species to date (Castro & Vari, 2004; Reis et al., 2016). In the Neotropical region, it is estimated that over 4 500 species inhabit its waters (Burns, 2021; Flecker, 1996; Wilson & Castro, 2010). This considerable diversity arises from evolutionary adaptations allowing them to exploit nutrient availability across various environments and trophic levels (Castro & Vari, 2004; Reis et al., 2016).

One of the most significant anatomical and physiological adaptations in fish is their capability to consume detritus as a nutrient source (detritivorous fish) (Agostinho et al., 2008; Benedito et al., 2018; Bowen, 2022; Burns, 2021; Natale et al., 2025; Wilson & Castro, 2010). Detritus consists of solid residues resulting from the decomposition of organic matter (Benbow et al., 2020; Bowen, 2022) and plays a crucial role in the food chain by releasing nutrients during decomposition that can be utilized by various species of microorganisms, invertebrates, and fish (Bowen et al., 2006; Bowen, 2022).

Another adaptation in fish involves modifications in their gastrointestinal tract (GIT). Detritivores typically have a longer intestinal length compared to their stomach, while carnivores exhibit a shorter intestinal length (Morales-González, 2023; Osorio-Urtecho, 2018; Salinas-Torres, 2011). Additionally, the GIT of fish consists of four concentric layers: the mucosal layer, submucosal layer, muscular layer, and serosal layer, from innermost to outermost, respectively. The mucosal layer, which borders the GIT lumen, serves the function of providing physical, chemical, and immunological protection to the tissue (Cao & Wang, 2009; Genten et al., 2008; Vidal et al., 2020).

This protective role is achieved through the secretion of glycoproteins known as mucins by goblet cells, constituting the major fraction of mucus and forming a natural barrier on the epithelial surface (Koshio, 2015). Acidic and neutral mucins are secreted in the mucosal layer of the esophagus, stomach, and intestine. These present a protection function by trapping water-soluble particles and defending them against pathogens. Furthermore, they protect the epithelium from the pH of gastric juices and act as lubricants, facilitating food passage (Koshio, 2015; Rodrigues & Cargnin-Ferreira, 2017; Vidal et al., 2020).

The study of gastrointestinal tract's (GIT) characteristics has been of great interest in Neotropical fish research (Lasso et al., 2011), since it varies depending on the type of diet and the microenvironments in which the individuals develop (dos Santos et al., 2015). Investigations have been conducted on various fish species such as Blanquillo (*Sorubim cuspicaudus*) (Oviedo-Montiel, 2021), Escalar (*Pterophyllum scalare*) (Ramírez-Espitia et al., 2020), Dentón (*Megaleporinus muyscorum*) (Mosquera-Ramos & Gómez-Ramírez, 2024), among others. In the case of the Characiformes order, the Prochilontidae family comprises several species like *Prochilodus lineatus* (found in Argentina, Paraguay, and Uruguay) (Bowen, 2022), *Prochilodus reticulatus* (Venezuela) (Vega-Contreras et al., 2017), *Prochilodus nigricans* (in the Amazon Basin), and for the Magdalena-Cauca basin, the Sinú and Atrato rivers, the *P. magdalenae* (Mojica et al., 2012; WWF Colombia & AUNAP, 2020). These species are of considerable importance for artisanal and commercial fishing and food security in river-side communities.

Although this species holds ecological, social, and economic significance, there is still a lack of studies addressing its anatomy, histology, and histochemistry of the digestive tract, hindering the understanding of its nutritional and physiological processes (Vega-Contreras et al., 2017).

The assessment of morphometric parameters, such as length and weight, at both whole-body (Hedayati et al., 2020) and organ-specific levels (Ramírez-Espitia et al., 2020), along with the analysis of body indices such as the hepatosomatic index (Favero et al., 2022) and gonadosomatic index (Hasim et al., 2021), emerges as fundamental tools for determining the anatomical structure and health status of fish. These methodologies, complemented by histological and histochemical analyses, provide crucial information that can contribute to enhancing species knowledge, as well as aiding in the development of conservation strategies (Serrano-López et al., 2021), detection of contaminants in their tissues (Liu et al., 2023), and improvement of adaptation to commercial aquaculture environments (Abdel-Tawwab et al., 2015).

These body indices not only provide information about the general condition of the organism but are also intrinsically linked to the function and structure of the digestive tract. The condition factor (K) reflects the overall nutritional status, which directly indicates the digestive system's efficiency and ability to process food (Radkhah & Eagderi, 2015). The hepatosomatic index (HSI) is particularly relevant, as the liver plays a crucial role in nutrient metabolism and bile production -both essential components of digestion- (Favero et al., 2022). The gonadosomatic index (GSI), in turn, helps to assess how reproductive cycles may influence both the allocation of energy resources and the histological structure of the digestive tract (Hasim et al., 2021). Finally, carcass yield (CY) provides valuable information about body composition, which results from the efficiency of nutrient digestion and absorption (Cabrera-Páez et al., 2008). The integration of these indices with histological and histochemical analyses allows for a more comprehensive understanding of the structure–function relationship of the gastrointestinal tract (Hedayati et al., 2020; Ramírez-Espitia et al., 2020), especially in herbivorous/detritivorous species such as *P. magdalenae*, in which these processes are particularly specialized (Serrano-López et al., 2021; Liu et al., 2023; Abdel-Tawwab et al., 2015).

Based on the detritivorous/herbivorous feeding habits of *P. magdalenae*, this study hypothesized that the gastrointestinal tract would exhibit specific adaptations: an elongated intestine with an intestinal coefficient greater than 2.5 (Wilson & Castro, 2010), enhanced muscular development in the pyloric region for mechanical processing of detritus (Burns, 2021), and differential distribution of mucins along the digestive tract (Díaz et al., 2008). The study predicted neutral mucins would predominate in anterior regions for protection, while acidic mucins would be more abundant posteriorly to facilitate nutrient absorption (Genten et al., 2008; Vidal et al., 2020). These adaptations would reflect the species' feeding strategy through specialized structures for increased absorption surface area and protection against abrasion from ingested material (Bowen, 2022; Wilson & Castro, 2010).

Consequently, this study aims to increase the fundamental understanding of the gastrointestinal tract by delineating the morphological and histochemical attributes of the digestive tract wall and the mucous cells within the mucosal layer, thus advancing comprehension of the digestive processes in this species.

## MATERIAL AND METHODS

All animal handling procedures were conducted in compliance with the standards for laboratory animal use as outlined by the Committee on Care and Use of Laboratory Animals of the National Research Council (National Academies, USA), eighth edition (Albus, 2012) and were also authorized by Resolution 0955 of May 27, 2020, issued by AUNAP, granting research permission to Piscícola San Silvestre.

**Fish sampling:** A total of 22 adult bocachico individuals (5 males and 17 females) were collected from their natural habitat in the Sogamoso River, a tributary branch of the Magdalena River basin, and then transferred to the Piscícola San Silvestre (PSS). These specimens were strategically distributed for different analyses: 15 individuals for morphometric measurements and body indices calculation, 5 for detailed histological and histochemical analyses, and 2 for macroscopic photographic documentation. All specimens were maintained in captivity for a maximum of 3 days to prevent any physiological changes due to artificial feeding and to ensure that data represented their natural condition. Following a 24-hour quarantine period in filtered water and two salt baths (20 ppm for 30 seconds), they were relocated to land ponds at a density of one fish per square meter. Periodically, Nutrimon® fertilizers (triple 15-15-15, providing nitrogen, phosphorus, and potassium) were administered to maintain optimal levels of primary productivity (phyto and zooplankton). Additionally, they were supplemented with commercially balanced food for tilapia containing 34 % CP (1.0 % of biomass), distributed in two daily rations.

**Performance parameters / Morphometric relationships / Body indices:** In the morphometric characterization of *P. magdalenae*, 15 specimens were employed. Measurements of total length (TL), standard length (SL), and intestinal length (IL) in centimeters (cm) were recorded using a fish-measuring board. Furthermore, measurements of total weight

(TW), eviscerated weight (EW), and the individual weight of the following organs: esophagus, stomach, intestine, liver, and gonads, were taken in grams (g) using an OHAUS® EB series scale. Moreover, the liver, gonads, and gills were collected and weighed individually in order to calculate the somatic indices and to support further physiological and histological analyses

Additionally, the photographic registry was conducted utilizing an EOS Rebel T3i camera equipped with a Canon EF-S 18-55 mm f/4-5.6 IS STM lens to analyze anatomical features. Subsequently, based on these measurements, the following morphometric relationships were computed following the criteria established by (Cabrera-Páez et al., 2008; Day et al., 2014).

$$Condition\ factor\ (K) = \frac{Total\ Weight\ (TW)}{Total\ Length\ cubed\ (TL^3)} * 100$$

$$Intestinal\ coefficient\ (CI) = \frac{Intestinal\ Length\ (IL)}{Total\ Length\ (TL)}$$

$$Gonadosomatic\ index\ (GSI) = \frac{Gonad\ Weight\ (GW)}{Total\ Weight\ (TW)} * 100$$

$$Hepatosomatic\ index\ (HSI) = \frac{Liver\ Weight\ (LW)}{Total\ Weight\ (TW)} * 100$$

$$Carcass\ yield\ (CY) = \frac{Eviscerated\ Weight\ (EW)}{Total\ Weight\ (TW)} * 100$$

Furthermore, with the purpose of obtaining an alternative morphometric index to the intestinal coefficient, one not only dependent on body lengths, but we also employed the *Zihler* index as outlined in the publication by Day et al. (2014).

$$Zihler\ index\ (ZI) = \frac{Intestinal\ Length\ (IL)}{10 * \sqrt[3]{Total\ Weight}}$$

The determination of these morphometric and body indices was carried out to establish relationships between the general physiological state and the characteristics of the digestive tract. The condition factor (K) was calculated to evaluate the nutritional status of specimens, which provides information on the digestive system's effectiveness in obtaining nutrients (Agbugui & Oniye, 2013). The intestinal coefficient (IC) and Zihler index (ZI) were determined to quantify specific morphological adaptations related to herbivorous/detritivorous feeding habits. The hepatosomatic index (HSI) was included due to the close functional relationship between the liver and the digestive tract regarding metabolism and nutrient processing. The gonadosomatic index (GSI) was calculated to examine the possible influence of reproductive status on the distribution of energy resources and its impact on digestive structure and function. Finally, carcass yield (CY) was determined to indicate the efficiency in converting food to body biomass. These parameters together allow contextualizing the histological and histochemical findings within the general physiological state of the studied specimens. The integration of these indices with histological observations enables a more comprehensive analysis of how the digestive tract structure, including epithelial organization, mucin distribution patterns, and tissue specialization, correlates with overall physiological condition. For instance, variations in K and HSI can be associated with changes in mucin production and epithelial characteristics, while IC and ZI provide context for interpreting specialized adaptations in the mucosal layer related to nutrient absorption efficiency, particularly important in detritivorous species where extensive surface area and specialized mucin secretions are critical for optimal digestive function (Pereira et al., 2014).

**Histological and histochemical analyses:** For the morphostructural characterization of the gastrointestinal tract, 22 randomly sampled individuals were employed. They were anesthetized with 10 ppm eugenol and euthanized by immersion in an ice bath. Opercula were then removed, and the left lateral wall dissection was conducted. Samples of the gastrointestinal tract were fixed in 10 % buffered formalin at a ratio of 1:10 for 24 hours.

The tissues underwent dehydration in ethanol at sequential concentrations, followed by cleaning with toluene or an equivalent solvent. Subsequently, they were embedded in hot paraffin, molded, and stored at room temperature as blocks. Sections of 5 to 6 μm thickness were obtained using a Leica RM2125 RTS rotary microtome. Following standard procedures, these sections were subsequently stained with hematoxylin and eosin (H&E). The respective images were captured using an Olympus® CX21 optical microscope and a Basler® ACA5472-17UC COLOR digital camera to qualitatively evaluate the digestive system structure (Verma et al., 2020; Vidal et al., 2020).

To detect neutral mucins, we conducted Periodic Acid-Schiff (PAS) staining, and for acidic mucins, Alcian Blue (AB) staining at pH 1.0 and pH 2.5 was utilized (Vidal et al., 2020). Subsequently, the conjugated AB pH2.5 + PAS technique was applied to detect the association between neutral and acidic mucins. Similarly, the specific detection of epithelial-origin mucins and acidic mucins in the gastrointestinal tract was achieved through mucicarmine staining (Kumar & Kiernan, 2010). Finally, Masson's trichrome (MT) staining was carried out for the identification of connective tissue.

**Transmission Electron Microscopy (TEM):** Fragments of gastrointestinal tissue (4-5 mm) were extracted and immersed in 2.5 % buffered glutaraldehyde in PBS (Phosphate Buffered Saline) for fixation. Following fixation, the samples were post-fixed in 1 % osmium tetroxide and 3 % uranyl acetate, gradually dehydrated, and infiltrated with a plastic resin mixed with acetone (1:1) before embedding in SPURR resin (Electron Microscopy Sciences, Fort Washington, PA, USA). The embedded tissue blocks were then sectioned using a Sorvall MT2-B ultramicrotome. Moreover, semithin sections (1 µm) were stained with toluidine blue and examined to identify suitable areas for ultrathin sectioning (Graham & Orenstein, 2007). These identified areas were cut with a diamond blade to a thickness of 80 - 100 nm, resulting in a yellow-gold interference color, and placed on 200 mesh copper grids. Subsequently, the sections were contrasted with uranyl acetate and lead citrate, followed by examination and photography using a JEOL 1400 Plus transmission electron microscope at the Department of Pathology, Hospital Universitario Fundación Santa Fe de Bogotá.

**Statistical analysis:** For the study, descriptive statistics were used to characterize the sample regarding morphological and zootechnical parameters, body parameters and indices were expressed as mean ± STD (standard deviation) (Moreno et al., 2019).

**Histochemical analysis:** Histochemical findings were analyzed based on tissue staining intensity for each staining technique. Intensity levels were determined through qualitative inspection, and the staining intensity results were detailed in a distribution table of acidic and neutral mucins in the gastrointestinal tract, similar to previous studies where specific morphological and histochemical adaptations have been observed in the digestive tract mucosa of other species such as *Brycon amazonicus* (Vidal et al., 2020).

## RESULTS

**Macroscopic description of gastrointestinal tract morphology:** The gastrointestinal tract of *P. magdalenae* (Fig. 1) is an elongated digestive system comprising four distinct regions: the oropharyngeal cavity, the esophagus, the stomach, and the intestine. The esophagus is a medium-sized tubular segment, approximately 2 cm long, characterized by thick muscular walls. The stomach has a U-shaped configuration, featuring three discernible regions: cardiac, fundic, and pyloric (Fig. 1D). The initial segment corresponds to a tubular portion posterior to the esophagus, which undergoes dilation to form a short sac-like structure (middle-fundic region), followed by a longer portion with considerably thick walls and a prominent muscular tunic (muscular stomach-pyloric region). The intestine of *P. magdalenae* is a lengthy, tightly coiled structure that occupies nearly the entire celomic cavity (Fig. 1B, Fig. 1C), with spirals arranged in paired fashion and curved in a levorotatory way (Fig. 1A, Fig. 1D). The anterior intestine

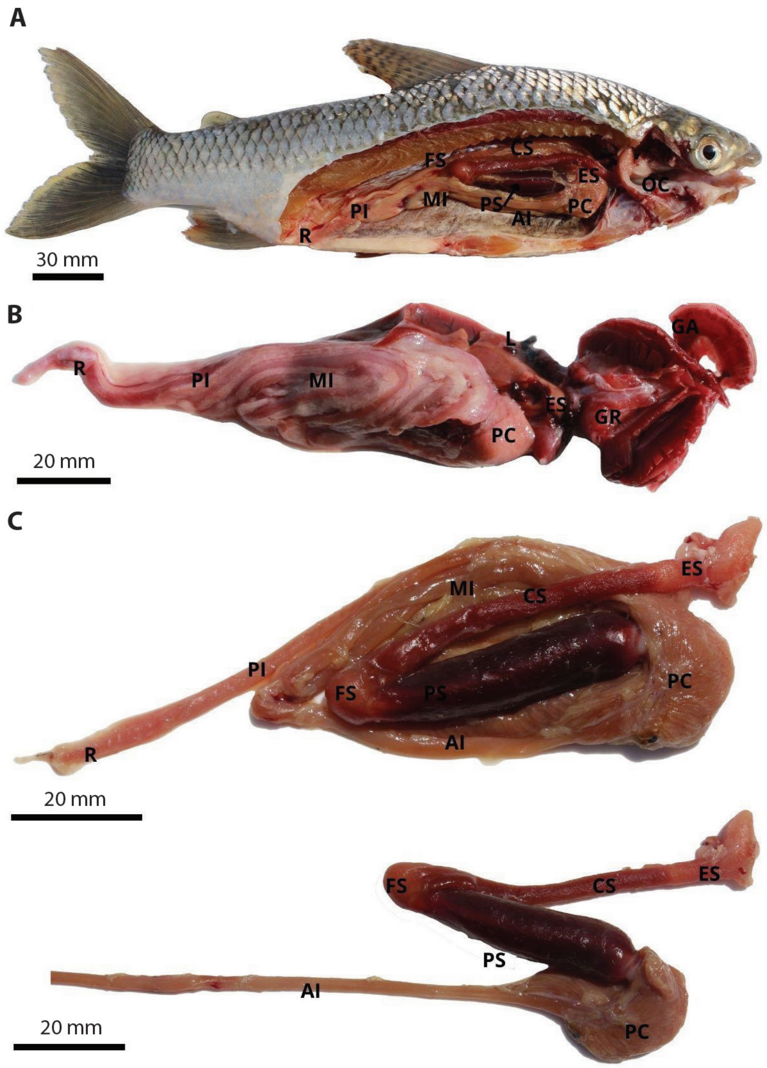

**Fig. 1. A.** Lateral view of the coelomic cavity of adult *P. magdalenae* showing the esophagus (Es), cardiac stomach region (CS), fundic stomach region (FS), pyloric stomach region (PS), pyloric ceca (PC), anterior intestine (AI), middle intestine (MI), posterior intestine (PI), and rectum (R). **B.** Anatomy of the digestive tract and cardiorespiratory organs of the oropharyngeal cavity displaying rectum (R), posterior intestine (PI), middle intestine (MI), pyloric ceca (PC), esophagus (Es), liver (L), gill arch (GA), and gill rakers (GR). **C.** Detailed anatomy of the gastrointestinal tract showing posterior intestine (PI), middle intestine (MI), cardiac stomach region (CS), esophagus (Es), fundic stomach region (FS), pyloric stomach region (PS), anterior intestine (AI), and pyloric ceca (PC).

exhibits a visibly larger diameter compared to the other segments, with thinner walls.

The morphometric measurements collected are detailed in Table 1, which presents the maximum, minimum, and average values with their respective standard deviations for all body parameters evaluated in the specimens studied.

The body indices, including the condition factor K, intestinal coefficient (IC), Zihler index (ZI), gonadosomatic index (GSI), hepatosomatic index (HSI), and carcass yield (CY), were calculated based on the data obtained from Table 1. Table 2 presents a summary of the estimated body indices.

**Table 1**
Body weights of 15 individuals of bocachico (*P. magdalenae*).

| | Maximum (g) | Minimum (g) | Average (g) |
|---|---|---|---|
| Total Weight | 440.0 | 221.0 | 291.77 ± 65.04 |
| Eviscerated Weight | 356.0 | 179.0 | 249.24 ± 58.06 |
| Intestine | 7.34 | 0.05 | 4.81 ± 1.31 |
| GIT | 32.02 | 2.54 | 12.02 ± 8.01 |
| Liver | 3.18 | 1.06 | 2.20 ± 0.47 |
| Gonads | 4.33 | 1.25 | 2.39 ± 1.02 |
| Bladder | 3.05 | 0.98 | 1.79 ± 0.60 |
| Stomach | 4.12 | 1.32 | 2.88 ± 0.55 |
| Esophagus | 1.26 | 0.11 | 0.60 ± 0.27 |

**Table 2**
Body indices of 15 individuals of bocachico (*P. magdalenae*).

| Index | Minium | Maximum | Average | Deviation |
|---|---|---|---|---|
| K | 0.84 | 1.18 | 1.02 | 0.09 |
| IC | 2.43 | 3.16 | 2.87 | 0.19 |
| ZI | 1.10 | 1.49 | 1.33 | 0.10 |
| GSI | 0.50 | 1.61 | 0.81 | 0.29 |
| HIS | 0.43 | 0.92 | 0.75 | 0.07 |
| CY | 65.28 | 94.80 | 85.20 | 7.78 |

Condition factor (K), intestinal coefficient (IC), *Zihler* index (ZI), hepatosomatic index (HSI), carcass yield (CY), and gonadosomatic index (GSI).

**Microscopic description of histology and histochemistry of the esophagus:** The average length of the esophagus in *P. magdalenae* was 1.26 ± 0.95 cm. In the anterior section, a mucosa covered initially by a non-keratinized stratified squamous epithelium was observed (Fig. 2B). However, as it progresses towards the middle region, digitiform folds protruding into the lumen are observed, lined by a simple columnar epithelium (ec), and again, stratified squamous epithelium is observed in the area near the stomach. Throughout the length of this organ, abundant mucous acinar glands are present. Below the basement membrane of the epithelium, a thick layer of loose connective tissue (tcl) was observed, corresponding to the lamina propria-submucosa (lp-sm), as no muscular layer of the mucosa was observed; therefore, there is no separation between the lamina propria and the submucosa (sm). Externally, the muscular layer, consisting of two layers of skeletal striated muscle, is observed, with the inner circular layer evident in the first third of the esophagus and the outer longitudinal layer which is thicker and more noticeable. Covering the muscular layer, an adventitia is observed as a thin layer of loose connective tissue.

Transmission electron microscopy (TEM) (Fig. 3) enabled a more detailed observation of the cellular morphology of the superficial layer of the epithelium, comprising thin cells with elongated central nuclei characteristic of a stratified squamous epithelium. These cells exhibit higher electron density than the goblet cells in the same tissue, and microvilli can also be observed on the apical surface of these cells (Fig. 3A, Fig. 3B). Additionally, large goblet cells containing mucin granules were observed, with some of these cells seen releasing part of these mucins into the lumen.

Through immunohistochemical staining, mucous acinar glands, some of them slightly oval-shaped, were observed (Fig. 2B, Fig. 2C). Staining with PAS, mucicarmine, AB pH 1.0,

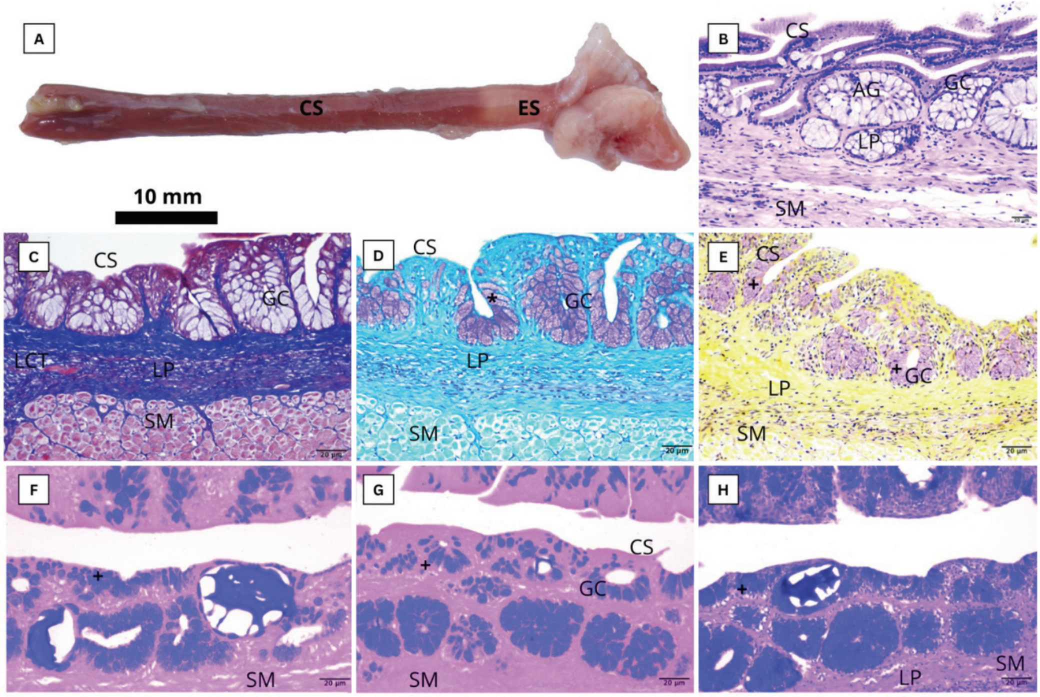

**Fig. 2**. Histological Organization and Dynamics of Mucins in the Middle Esophagus of *P. magdalenae*. **A.** Detailed anatomy of the esophagus showing esophagus (ES) and cardiac stomach region (CS). The esophageal epithelium appears as a simple columnar epithelium (EC) with scattered goblet cells (GC), overlaying the basement membrane and the lamina propria (LP) composed of loose connective tissue (LCT). The tissue also contains acinar glands (AG) visible in the submucosal region, and a layer of smooth muscle (SM) underlying all structures. Goblet cells exhibit the presence of neutral mucins (marked with *) and acidic mucins (marked with +), as observed in staining descriptions: **B.** Simple columnar epithelium with scattered goblet cells, accompanied by numerous longitudinal folds towards the lumen, increasing the absorptive surface. Thin lamina propria and thick muscular layer (H&E 100x). **C.** Simple columnar epithelium with goblet cells, showing a broad lamina propria with abundant, blue-stained loose connective tissue (MT 40x). **D.** Numerous goblet cells with intensely magenta-stained apical portions, indicating a high presence of neutral mucins (PAS 40x). **E.** Goblet cells with intensely blue-stained apical portions, revealing significant amounts of acidic mucins (Mucicarmine 40x). **F.** Intense blue staining in goblet cells, demonstrating a high concentration of sulphated and carboxylated acidic mucins (AB pH 2.5 40x). **G.** Slight blue staining in some goblet cells, indicating a low presence of sulphated acidic mucins (AB pH 1.0). **H.** Combination allowing the distinction of goblet cells with neutral mucins (magenta) and sulfated/carboxylated acidic mucins (blue) (PAS/AB pH 2.5).

and AB pH 2.5 facilitated the visualization of neutral mucins (Fig. 2D, Fig. 2E, Fig. 2F, Fig. 2G). Staining for sulfated and carboxylated acidic mucins was exclusively present in mucus cells, while labeling for neutral mucins was observed in both mucus and epithelial columnar cells. Co-localization of acidic and neutral mucins was observed with PAS/AB pH 2.5 staining (Fig. 3H), with acidic mucins predominating.

**Histology and histochemistry of the stomach:** The initial segment displayed a wide mucosal layer characterized by numerous short folds projecting towards the lumen. It was lined by a simple columnar epithelium (SCE) and featured abundant tubular gastric glands containing oxyntic peptic cells. Sparse loose connective tissue was observed between the glands, corresponding to the lamina propria (Fig. 4B, Fig. 4C). A thin layer of smooth muscle, corresponding to the muscularis mucosae, was evident towards the end of the mucosal layer.

Additionally, a thin layer of loose connective tissue (submucosal layer) was observed externally to the mucosal layer. Below this, two

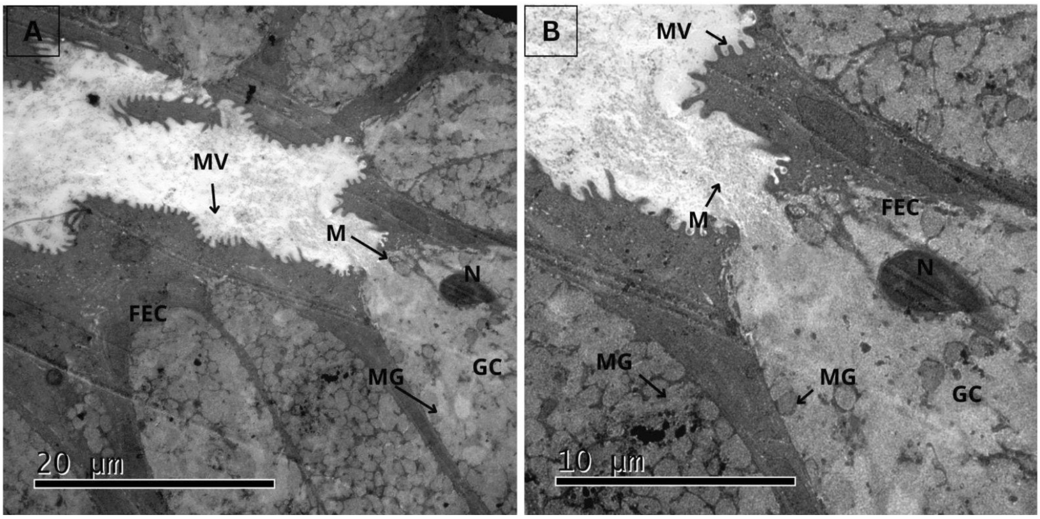

**Fig. 3.** Transmission electron microscopy (TEM) of the esophagus of *P. magdalenae* reveals: **A.** Flat epithelial cells (FEC) with microvilli (MV), goblet cells (GC), and mucin granules (MG) inside the goblet cell, with (M) representing mucin release into the lumen. The nucleus is labeled as (N). **B.** Mucin release (M) and mucin granules (MG) in the cytoplasmic region of the cell are observed in greater detail, with (N) indicating the nucleus.

layers of skeletal striated muscle, consisting of an inner circular layer and an outer longitudinal layer (muscular layer), were noted. Moreover, an outer layer of loose connective tissue, known as the serosal layer, was observed externally (Fig. 4C).

Furthermore, macroscopically, the central section appears as a short sac-like structure (Fig. 4A), representing a transitional zone where the mucosal layer thins out. It is lined by a simple columnar epithelium, under which lies a thin layer of loose connective tissue (lamina propria), accompanied by blood vessels, some mucosal folds, and gastric pits. This mucosa is thinner than the preceding one, attributable to the absence of glands with oxyntic peptic cells cells. Posterior to the lamina propria, the mucosal layer exhibited a thin layer of smooth muscle corresponding to the muscularis mucosae. Beneath the mucosal layer, a layer of connective tissue corresponding to the submucosal layer, composed of loose connective tissue (LCT), was observed, with the muscular layer located externally to it. In this segment of the stomach, the muscular layer displays up to three layers of smooth muscle: an inner oblique layer, a middle circular layer, and an outer longitudinal layer. Beyond the muscular layer, a thin layer of loose connective tissue representing the serosal layer was identified.

In the observations made using TEM (Fig. 5), epithelial cells with abundant granules exhibiting pronounced electron density towards the apical zone were observed. Additionally, intraepithelial cells, possibly lymphocytes migrating through the epithelium, were noted within the epithelial layer. Furthermore, invaginations were observed, contributing to the epithelium’s lobulated appearance.

The third gastric segment corresponded to a region with a thick muscular wall (Fig. 6A), consisting of a mucous membrane lined by a simple columnar epithelium and abundant simple straight tubular glands. Beneath the epithelium, there was a thin layer of loose connective tissue, either the lamina propria or submucosa, as no muscular layer of the mucosa separating the lamina propria from the submucosa was observed. Externally to this, there was a very thick layer of smooth muscle corresponding to the muscularis externa, which was the widest layer in this segment of the digestive tract. External to the muscularis externa, there was a thin layer of connective tissue corresponding

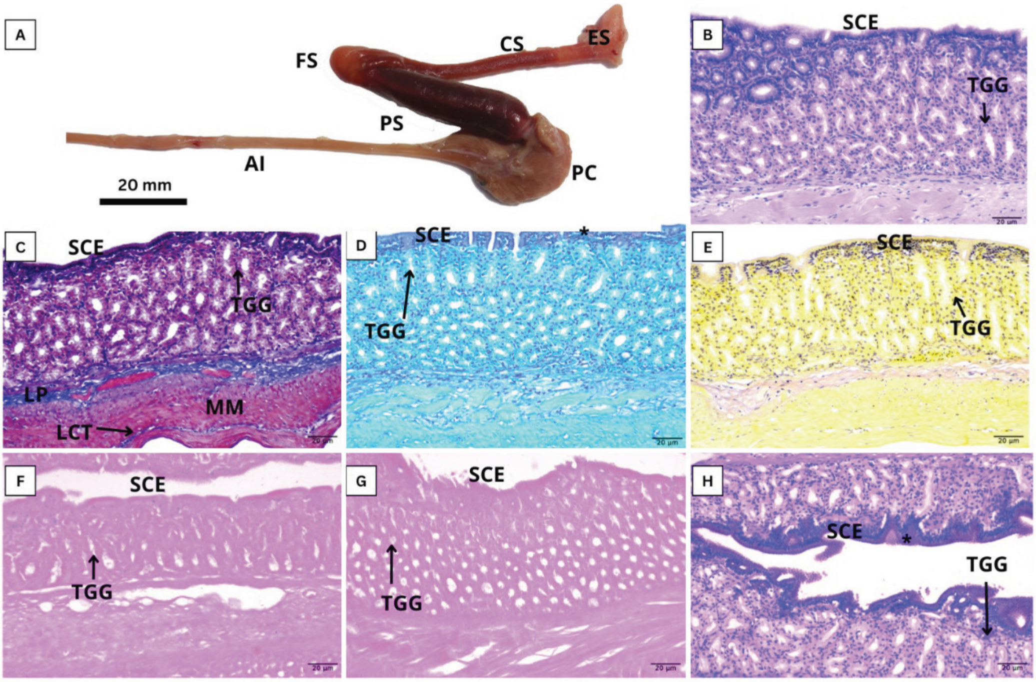

**Fig. 4.** Histological organization and mucin dynamics in the stomach of *P. magdalenae*. **A.** Detailed anatomy of the gastrointestinal tract showing posterior intestine (PI), middle intestine (MI), cardiac stomach region (CS), esophagus (Es), fundic stomach region (FS), pyloric stomach region (PS), anterior intestine (AI), and pyloric ceca (PC). **B.** Section showing a simple columnar epithelium (SCE) and numerous tubular gastric glands (TGG), H&E staining, 100x. **C.** Section displaying the lamina propria (LP) composed of loose connective tissue (LCT), MT staining, 40x. **D.** Section showing the presence of neutral mucins (*), PAS staining, 40x. **E.** Section showing complete absence of acidic mucins, Mucicarmine staining, 40x. **F.** Section displaying complete absence of acidic mucins, AB pH 2.5 staining, 40x. **G.** Section showing complete absence of acidic mucins, AB pH 1.0 staining, 40x. **H.** Section displaying the presence of neutral mucins (*) and absence of acidic mucins, PAS/AB pH 2.5 staining, 40x. Abbreviations: posterior intestine (PI), middle intestine (MI), cardiac stomach region (CS), esophagus (ES), fundic stomach region (FS), pyloric stomach region (PS), anterior intestine (AI), pyloric ceca (PC), simple columnar epithelium (SCE), tubular gastric glands (TGG), lamina propria (LP), loose connective tissue (LCT), Hematoxylin and Eosin (H&E), Masson's Trichrome (MT), Periodic Acid-Schiff (PAS), and Alcian Blue (AB).

to the serous membrane. Mucin dynamics in all stomach regions exclusively corresponded to neutral mucins.

**Histology and histochemistry of the intestine:** The average length of the intestine in *P. magdalenae* was 88.18 ± 7.35 cm. Histologically, four segments can be distinguished: anterior, glandular, middle, and posterior. All segments exhibit a mucosal tunica with digitiform folds projecting into the lumen, with shorter folds in the posterior segment. The mucosal tunica in all segments is lined by a simple columnar epithelium, composed of enterocytes and goblet cells. In the posterior segment, there is a reduction in the number of columnar cells and an increase in mucus-producing cells. Beneath the epithelium in the anterior, middle, and posterior segments, a layer of loose connective tissue corresponding to the lamina propria-submucosa was observed, as no muscularis mucosae separating these layers from the connective tissue was found. Additionally, externally, two layers of smooth muscle -the inner circular and the outer longitudinal- were observed, forming the muscular tunica.

External to the muscular tunica, there is a thin layer of loose connective tissue

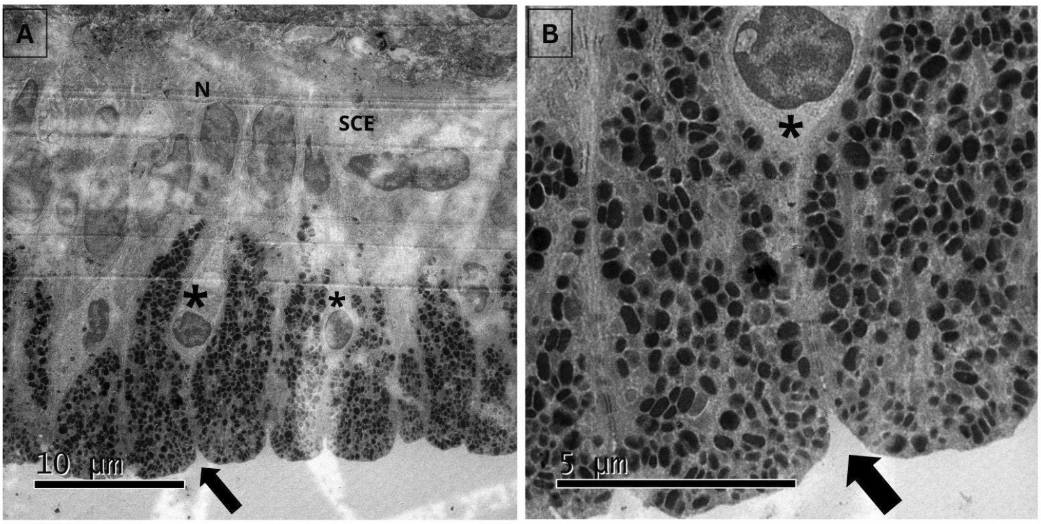

**Fig. 5.** Ultrastructural analysis of the gastric cardiac region epithelium of *P. magdalenae*. Transmission electron micrographs of the gastric cardiac region showing: **A.** Simple columnar epithelium (SCE) with intraepithelial cells (*), epithelial invagination (↑), and nuclei (N), scale bar: 10 µm. **B.** Higher magnification view of the epithelium displaying secretory granules and an intraepithelial cell (*), with epithelial surface indicated by arrow (↑), scale bar: 5 µm.

corresponding to the serosal tunica. The anterior segment of the intestine opens into multiple finger-like structures that terminate blindly, corresponding to the pyloric caeca with a mucosal tunica exhibiting digitiform folds projecting into the lumen, lined by a simple columnar epithelium (Fig. 7B, Fig. 7C). Beneath this layer lies a thin layer of loose connective tissue, the lamina propria, and externally, the muscular tunica with two layers of smooth muscle, the inner circular and the outer longitudinal, and peripherally, a thin layer of loose connective tissue, the serosal tunica. Posterior to the anterior segment of the intestine in this species, there is an area where the mucosal tunica, unlike the other regions of the intestine, does present a muscular layer of the mucosa, separating the lamina propria from the submucosal tunica, with abundant acinar glands producing mucus in the latter. Beyond the submucosal tunica, the muscular and serosal tunicas are similar to those reported in the other segments.

When evaluating the ultrastructure of the intestinal epithelium across various segments using Transmission Electron Microscopy (TEM) (Fig. 8), a greater abundance of enterocytes is evident in the anterior and middle regions. The presence of a simple columnar epithelium is confirmed, characterized by abundant microvilli (brush border) prominently displayed on the apical surface of the epithelial cells.

Histochemical analysis revealed the presence of neutral and sulfated acidic mucins, as well as carboxylated acidic mucins, with moderate intensity in the anterior and middle segments, attributable to the low number of mucus cells in these areas. A slight predominance of neutral mucins was observed in the anterior portion, whereas in the middle and posterior segments, there was a predominance of acidic mucins, primarily carboxylated (Fig. 9). Table 3 summarizes the observed dynamics of acidic and neutral mucins in the digestive tract of *P. magdalenae*, also emphasizing crucial histological aspects of this species regarding the types of cells involved in mucin secretion.

## DISCUSSION

The Fulton condition factor (K) is an index utilized to assess the impact of diverse

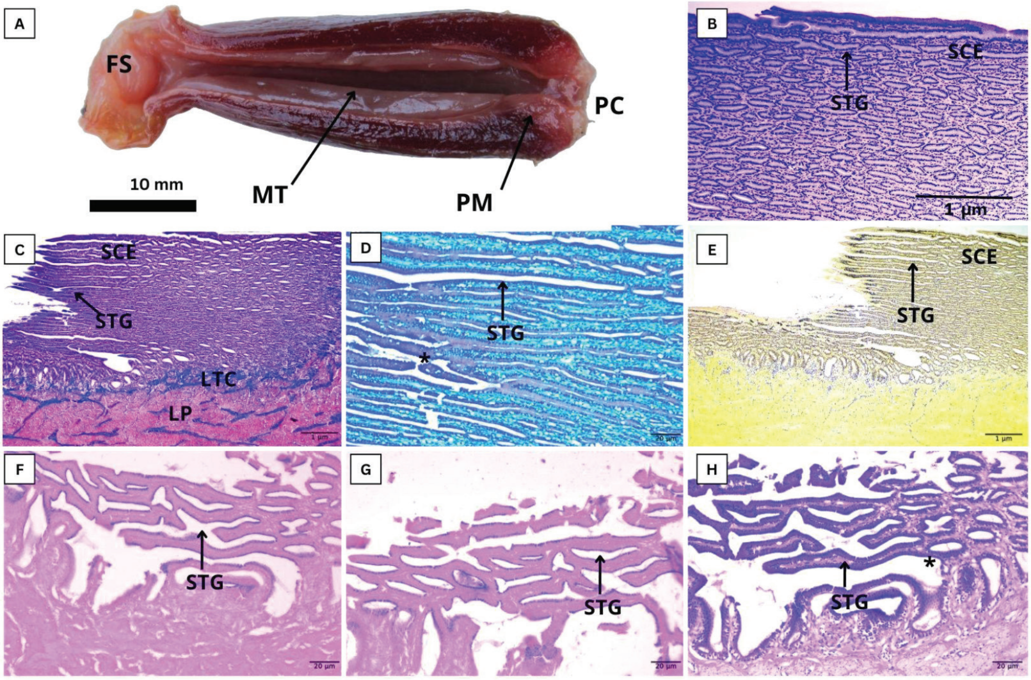

**Fig. 6.** Histological and dynamic organization of mucins in the pyloric region of the stomach of *P. magdalenae*. **A.** Macroscopic view showing the presence of a prominent muscular wall (PM) and a mucosal tunica (MT), which delineate the fundic stomach (FS) and its connection with the pyloric caeca (PC). Scale bar: 10 mm. **B.** Microscopic view of the mucosa lined with a simple columnar epithelium (SCE) and simple straight tubular glands (STG). H&E staining, 10x. Scale bar: 1 µm. **C.** The detail shows a simple columnar epithelium (SCE), simple straight tubular glands (STG), and the presence of loose connective tissue (LTC), specifically the lamina propria (LP). MT staining, 10x. **D.** The section shows simple straight tubular glands (STG) and the presence of neutral mucins (indicated by *). PAS staining, 40x. **E.** Section displaying simple columnar epithelium (SCE) and simple straight tubular glands (STG) with complete absence of acidic mucins. Mucicarmine staining, 10x. Scale bar: 1 µm. **F.** Section showing simple straight tubular glands (STG) with complete absence of acidic mucins. AB pH 2.5 staining, 40x. Scale bar: 20 µm. **G.** Section displaying simple straight tubular glands (STG) with complete absence of acidic mucins. AB pH 1.0 staining, 40x. Scale bar: 20 µm. **H.** Section showing simple straight tubular glands (STG) with the presence of neutral mucins (indicated by *) and absence of acidic mucins. PAS/AB pH 2.5 staining, 40x. Scale bar: 20 µm. Abbreviations: prominent muscular wall (PM), mucosal tunica (MT), fundic stomach (FS), pyloric caeca (PC), simple columnar epithelium (SCE), simple straight tubular glands (STG), loose connective tissue (LTC), lamina propria (LP), Hematoxylin and Eosin (H&E), Masson's Trichrome (MT), Periodic Acid-Schiff (PAS), Alcian Blue (AB).

environmental conditions on fish health, particularly in relation to their nutritional status. K values exceeding 1.0 are indicative of favorable environmental and feeding conditions conducive to fish development, whereas values below 1.0 suggest less favorable conditions (Radkhah & Eagderi, 2015). In this study, the K factor was computed to evaluate the well-being of captured individuals, yielding an average of 1.02 ± 0.09. This value is notably lower than those reported by Vega-Contreras et al. (2017) at three distinct sites along the Sogamoso River, where average values of 1.21 ± 0.03, 1.24 ± 0.06, and 1.20 ± 0.05 were documented. Contreras-Almazo et al. (2019) contend that the heightened presence of anthropogenic debris may have precipitated the premature sexual maturation of *P. magdalenae*, a phenomenon previously observed in *P. lineatus* and associated with K factor values surpassing 1.0 (Alonso et al., 2015; Cañas-Alva et al., 2020; Cifuentes et al., 2012). Potential explanations for the variance in K values

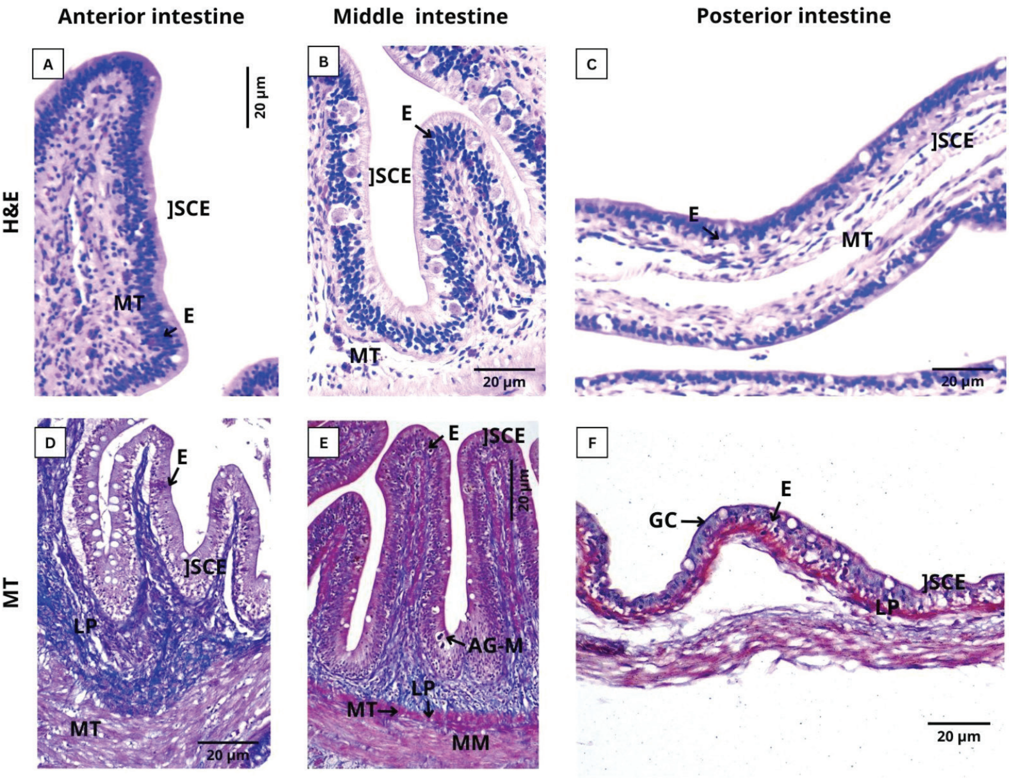

**Fig. 7.** Histology of the intestine of *P. magdalenae*. The histological organization of the intestine of *P. magdalenae* is characterized by: **A.** Anterior intestine section showing a simple columnar epithelium (JSCE) with enterocytes (E) and the muscular tunica (MT), H&E staining, 40x. **B.** Middle intestine section showing a simple columnar epithelium (JSCE) with enterocytes (E) and the muscular tunica (MT), H&E staining, 40x. **C.** Posterior intestine section showing a simple columnar epithelium (JSCE) with enterocytes (E) and the muscular tunica (MT), H&E staining, 40x. **D.** Anterior intestine section revealing a simple columnar epithelium (JSCE) with enterocytes (E), the lamina propria (LP), and the muscular tunica (MT), MT staining, 40x. **E.** Middle intestine section displaying a simple columnar epithelium (JSCE) with enterocytes (E), lamina propria (LP), mucosa/muscularis (MM), and acinar glands-mucin (AG-M), MT staining, 40x. **F.** Posterior intestine section displaying a simple columnar epithelium (JSCE) with goblet cells (GC), enterocytes (E), and the lamina propria (LP), MT staining, 40x. Scale bars: 20 µm. Abbreviations: simple columnar epithelium (JSCE), enterocytes (E), goblet cells (GC), lamina propria (LP), muscular tunica (MT), mucosa/muscularis (MM), acinar glands-mucin (AG-M), Hematoxylin and Eosin (H&E), Masson's Trichrome (MT).

between this study's findings for *P. magdalenae* and those of Contreras-Almazo et al. (2019), encompass factors such as the fish's residence in ponds supplied with tributary water that has undergone prior filtration, seasonal disparities, and the controlled feeding regimen employed within the San Silvestre fishery (Ragheb, 2023).

The morphometric indices analyzed in this study provide valuable context for interpreting the histological and histochemical findings in *P. magdalenae*'s digestive tract. The condition factor (K) of 1.02 ± 0.09 indicates adequate but not optimal nutritional status, which directly influences the efficiency of the digestive system and potentially affects mucin production patterns observed in the different GIT segments (Kroon et al., 2017). This K value, while sufficient for normal physiological function, is lower than previously reported values for this species (Vega-Contreras et al., 2017), suggesting potential differences in digestive efficiency that align with our histochemical observations

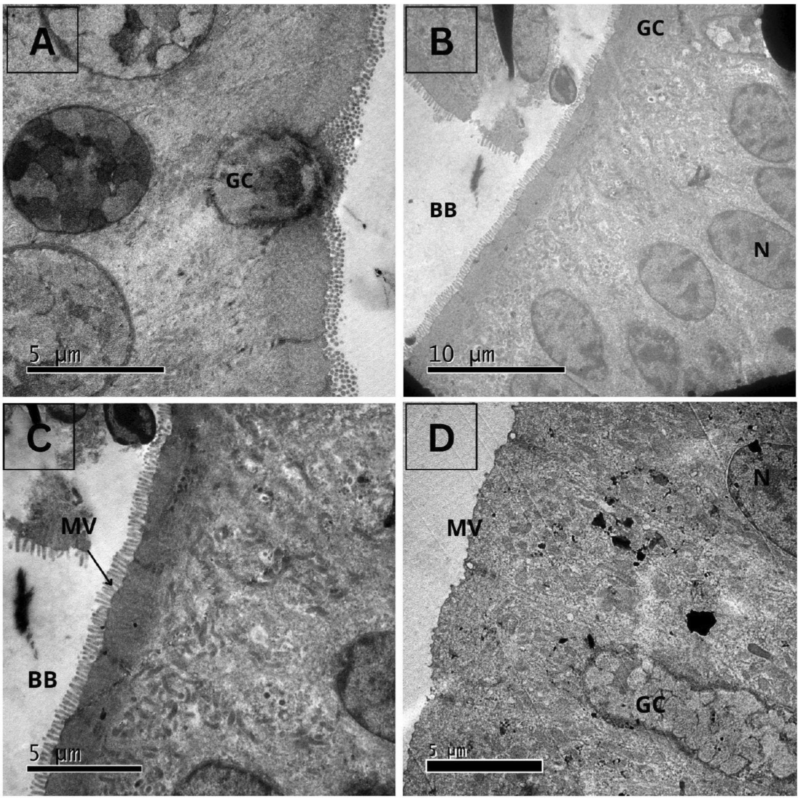

**Fig. 8.** Epithelial ultrastructure of the intestine of *P. magdalenae*. Transmission electron micrographs showing the ultrastructural organization of the intestinal epithelium of *P. magdalenae* as follows: **A.** Anterior intestine showing goblet cells (GC) with secretory granules, scale bar: 5 µm. **B**. Middle intestine displaying goblet cells (GC), the brush border of the intestinal epithelium (BB), and enterocyte nuclei (N), scale bar: 10 µm. **C.** Middle intestine section showing the brush border (BB) with well-developed microvilli (MV), scale bar: 5 µm. **D.** Posterior intestine displaying microvilli (MV), goblet cells (GC), and nuclei (N), scale bar: 5 µm. Abbreviations: GC: goblet cells; MV: microvilli; N: nucleus; BB: brush border.

of mucin distribution. The average hepatosomatic index (HSI) of the fish in the study was 0.75 ± 0.07. Considering that HSI values below 1.0 are associated with low energy reserves, which may be linked to situations such as reduced food availability, environmental stress, presence of contaminants, or the onset of the reproductive stage (Cabrera Páez et al., 2008), the observed low energy reserves in the fish suggest potential preparation for the reproductive stage or conditions in captivity that differ from their natural environment. Factors such as food quality and availability, as well as physicochemical water conditions, may contribute to these differences (Contreras-Almazo et al., 2019; Mancera-Rodríguez et al., 2016), because in natural environments, food availability is often linked to the presence of organic matter and macrophytes (Mojica et al., 2012). The hepatosomatic index (HSI) of 0.75 ± 0.07 is particularly relevant for digestive function interpretation, as the liver plays a crucial role in bile production -essential for lipid digestion- and detoxification of compounds from the digestive tract (Figueiredo-Silva et al., 2013). This relatively low HSI value suggests limited energy reserves, which may influence the specialized mucin secretion patterns we observed, particularly the predominance of carboxylated acidic mucins in the posterior intestinal segments that

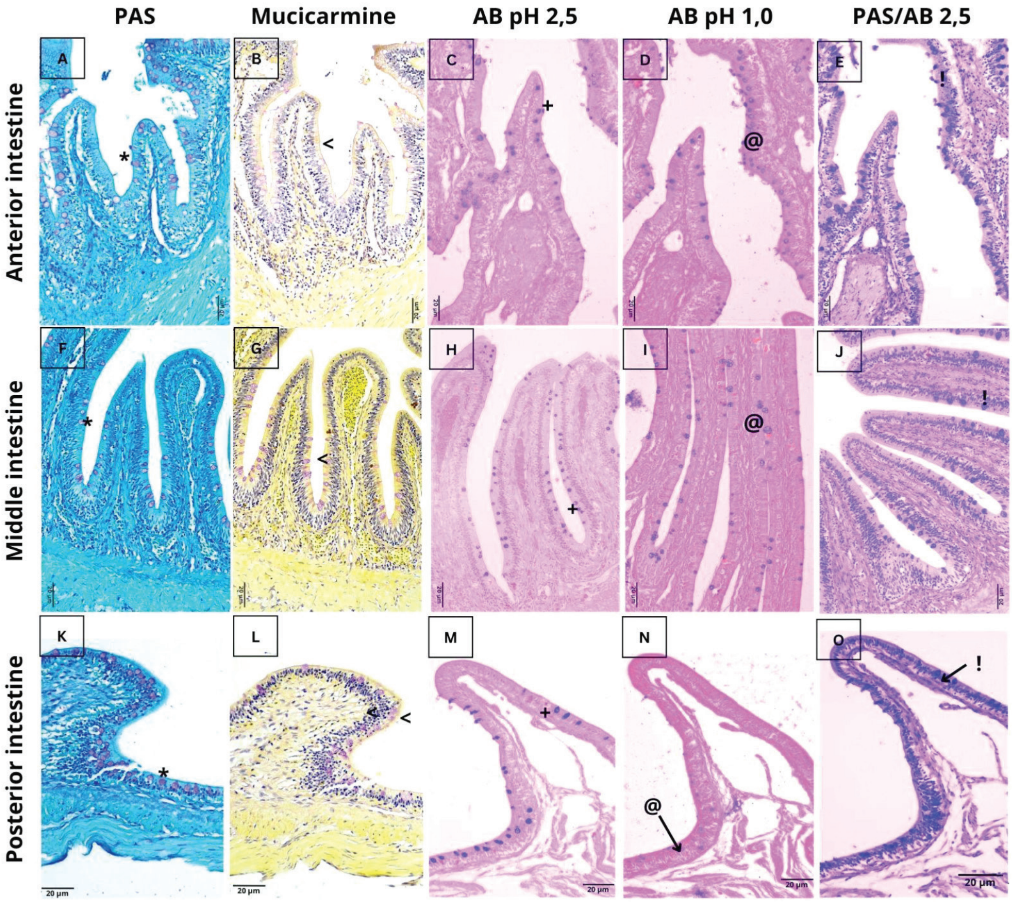

**Fig. 9.** Mucin histochemistry of the intestine of *P. magdalenae*. The histochemical characterization of mucins in the intestine of *P. magdalenae* is presented as follows: **A.** Anterior intestine showing the presence of neutral mucins (*) in goblet cells, PAS staining, 40x. **B.** Anterior intestine displaying epithelial acidic mucins (<) in goblet cells, Mucicarmine staining, 40x. **C.** Anterior intestine showing carboxylated acidic mucins (+), AB pH 2.5 staining, 40x. **D.** Anterior intestine showing sulfated acidic mucins (@), AB pH 1.0 staining, 40x. **E.** Anterior intestine displaying colocalization of acidic and neutral mucins (!), PAS/AB pH 2.5 staining, 40x. F. Middle intestine showing the presence of neutral mucins () in goblet cells, PAS staining, 40x. **G.** Middle intestine displaying epithelial acidic mucins (<) in goblet cells, Mucicarmine staining, 40x. **H.** Middle intestine showing carboxylated acidic mucins (+), AB pH 2.5 staining, 40x. I. Middle intestine showing sulfated acidic mucins (@), AB pH 1.0 staining, 40x. **J.** Middle intestine displaying colocalization of acidic and neutral mucins (!), PAS/AB pH 2.5 staining, 40x. **K.** Posterior intestine showing the presence of neutral mucins (*) in goblet cells, PAS staining, 40x. **L.** Posterior intestine displaying epithelial acidic mucins (<) in goblet cells, Mucicarmine staining, 40x. **M.** Posterior intestine showing carboxylated acidic mucins (+), AB pH 2.5 staining, 40x. **N.** Posterior intestine showing sulfated acidic mucins (@), AB pH 1.0 staining, 40x. **O.** Posterior intestine displaying colocalization of acidic and neutral mucins (!), PAS/AB pH 2.5 staining, 40x. Scale bars: 20 µm. Abbreviations: Periodic Acid-Schiff (PAS), and Alcian Blue (AB).

enhance nutrient absorption efficiency (Caballero et al., 2004).

The gonadosomatic index (GSI) of 0.81 ± 0.29 indicates potential reproductive activity that affects nutrient allocation within the organism. During reproductive periods, resources may be diverted from digestive processes to gonadal development, potentially altering mucin production patterns and the histological structure of the digestive tract (Zaldúa & Naya, 2014). This relationship between reproductive status and digestive histology requires

**Table 3**
Dynamic of acidic and neutral mucins in the digestive tract of *P. magdalenae*.

| Staining | Type of mucins | Esophagus – Cranial portion | Esophagus – Middle portion | Esophagus – hind portion | Stomach – Antral region | Stomach – Fundic region | Stomach – Pyloric region | Foregut | Midgut | Hindgut |
|---|---|---|---|---|---|---|---|---|---|---|
| PAS | Neutral mucins | +++ GC and epithelium | +++<br>GC and epithelium | +++<br>GC and epithelium | +++<br>epithelium | +++<br>epithelium | +++<br>Epithelium | ++<br>Few GC | + | ++ |
| AB pH 1.0 | Sulfated acidic mucins | +++ GC | +++ GC | +++ GC | - | - | - | ++<br>Few CC | ++ | ++ |
| AB pH 2.5 | Carboxylated acidic mucins | +++ GC | +++ GC | +++ GC | - | - | - | ++<br>Few CC | ++ | ++ |
| PAS/AB pH 2.5 | Acid and neutral mucins | +++ predominance of acidic mucins and slight neutral marking | +++ predominance of acidic mucins and slight neutral marking. EC exclusively neutral dialing | +++ predominance of acidic mucins and slight neutral marking. EC exclusively neutral dialing | +++<br>exclusively neutral | +++<br>exclusively neutral | +++<br>exclusively neutral | ++<br>Few GCs, both acidic and neutral (slightly predominant) | ++<br>Predominance of carboxylated acidic mucins | ++<br>Predominance of carboxylated acidic mucins |
| Mucicarmin | Epithelial acidic mucins | ++ GC | ++ GC | ++ GC | - | - | - | + | + | ++ |

The intensity of staining was assessed as (–) absence, (+) mild, (++) moderate and (+++) intense, (GC) goblet cells.

further investigation in *P. magdalenae*. Finally, the carcass yield (CY) of 85.20 ± 7.78 reflects overall body composition and provides indirect evidence of digestive efficiency, as higher values indicate better conversion of ingested nutrients into body mass (Salem et al., 2018). The CY values observed support our findings regarding the specialized intestinal structure with its elongated form (intestinal coefficient of 2.87 ± 0.19) and differential mucin distribution that maximizes nutrient extraction from detrital material (Jiao et al., 2023). Together, these indices provide a comprehensive physiological context that enhances our understanding of the structure-function relationship in the digestive tract of this detritivorous species.

Further analysis of the K factor (1.02 ± 0.09) and HSI (0.75 ± 0.07) values requires consideration of multiple environmental and physiological variables that could influence these indices (Mozsár et al., 2015). The controlled food availability in the San Silvestre fishery, with supplementary balanced feed (34 % CP) administered at 1.0 % of biomass, differs significantly from the natural feeding conditions of wild *P. magdalenae* populations that primarily consume detritus and periphyton. This difference in diet composition and availability could explain the lower K values compared to those reported by Vega-Contreras et al. (2017). Similarly, the relatively low HSI values observed may reflect an adaptation to captive conditions with controlled feeding regimens rather than energy deficiency per se. Population density also plays a crucial role in modulating these indices, as the experimental density of one fish per square meter represents a significantly less crowded environment than natural conditions, potentially reducing competition stress but also altering feeding behavior (Abdel-Tawwab et al., 2015). Seasonal variables and reproductive cycles further interact with these indices, as demonstrated by Cifuentes et al. (2012) and Cañas-Alva et al. (2020) in related species. The relationship between reproductive status and both K and HSI is particularly relevant, as energy reserves are redistributed during gonadal development. The GSI values (0.81 ± 0.29) in our specimens suggest that some individuals were in early gonadal development stages, which may have influenced both hepatic reserves and overall body condition (Nunes et al., 2011). These interrelated factors -food availability, population density, and reproductive status -collectively impact the digestive physiology observed in our histological and histochemical analyses, particularly the mucin distribution patterns that optimize nutrient absorption under specific energetic demands.

On the other hand, Karachle and Stergiou (2010) argue that a strong correlation exists between intestinal length and the feeding habits of fish species. Typically, carnivorous species possess shorter intestines, while omnivores, herbivores, and detritivores tend to have longer ones. Similarly, morphometric parameters concerning intestinal length, such as the intestinal coefficient (CI) and Zihler's index, are frequently utilized to deduce the feeding behaviors of fish species. In this study, the estimated values for both indices were 2.87 ± 0.19 and 1.33 ± 0.10, respectively. The CI aligns with what is reported for omnivorous species but is notably close to the value of 3.0 specified for detritivores (Karachle & Stergiou, 2010). Concerning *Zihler*'s index (IZ), which establishes a morphometric relationship between intestinal length and fish body mass, it delineates the following ranges: pure herbivores (2.0 to 2.1), omnivores with a herbivorous inclination (2.1 to 2.9), omnivores with an animal inclination (2.9 to 3.7), carnivores with a preference for decapods and fish (3.7 to 4.0), and carnivores with a preference for fish and cephalopods (4.0 to 4.5) (Day et al., 2014; Karachle & Stergiou, 2010). The estimated IZ value for *P. magdalenae* falls below 2, indicating a classification as a pure herbivore within this system; nevertheless, there is an insufficient number of reports for detritivore classification and IZ values tailored to this feeding habit.

The morphology and observed distribution of the gastrointestinal system organs in *P. magdalenae* align with the compiled information on the generalities and specificities of digestive structures in teleosts (Genten et al., 2008; Wilson & Castro, 2010). *P. magdalenae* possesses a lengthy digestive tract comprised of four well-defined regions (oropharyngeal cavity, esophagus, stomach, and intestine) similar a *B. amazonicus* (Vidal et al., 2020), *Schizodon knerii* (dos Santos et al., 2015), and other, more distantly related taxa such as *Tilapia sparrmani* (Okuthe & Bhomela, 2021), and *Oreochromis niloticus* and *Clarias gariepinus* (Awaad et al., 2014).

The secretion of neutral mucins in the mucosa of the esophageal tract facilitates food passage while providing protection against abrasion damage due to the absence of saliva and a masticatory process, as seen in other vertebrates. Additionally, acidic mucins offer protection against the establishment of potential pathogens in the esophageal lumen, and contribute to food degradation before it enters the stomach (Awaad et al., 2014; Cornick et al., 2015; Firmino et al., 2020; Marchetti et al., 2006; Phrompanya et al., 2019).

Glycoproteins, particularly mucins secreted by goblet cells throughout the digestive tract of *P. magdalenae*, play crucial roles in epithelial protection against abrasion from organic matter (Nachi et al., 1998). The abundance of acinar glands in the esophagus that produce both acidic and neutral mucins forms a protective barrier that shields the epithelium from mechanical damage caused by ingested sediments, common in detritivorous feeding (Cyrino et al., 2008). This mucin layer acts as a renewable physical barrier preventing direct contact between abrasive sediment particles and delicate epithelial surfaces, reducing cellular damage and irritation, especially in the pyloric region which functions similarly to a gizzard in the mechanical processing of detritos (Baldisserotto et al., 2019).

The differential distribution of mucins facilitates both protection and efficient digestion of particulate organic matter, with neutral mucins predominating in anterior regions (esophagus and anterior intestine) and acidic mucins in middle and posterior intestinal regions Acidic mucins, especially abundant in the middle and posterior intestine, help trap organic particles from detritus, increasing contact time with digestive enzymes and enhancing nutrient extraction efficiency (Palladino et al., 2023). Additionally, mucins may create microenvironments that optimize the function of digestive enzymes needed to break down organic matter in detritus, which often contains complex carbohydrates and other nutrients embedded in difficult-to-digest matrices, representing a key evolutionary adaptation that allows *P. magdalenae* to efficiently exploit detrital food resources (Ortiz-Ruiz et al., 2024).

In terms of ultrastructure, similarities are observed in the arrangement of acinar glands, the presence of microvilli towards the luminal part, interruption of the continuity of squamous epithelial tissue, and abundant granular content of mucins when compared with species such as *Saliminus affinis* (Atencio-García et al., 2008), *Sparus aurata* (Abumandour & El-Bakary, 2018) and *Anguilla anguilla* (Knutsen et al., 2021). For the genus *Prochilodus*, there are no studies supporting findings at the esophageal structure level. The stomach of *P. magdalenae*, due to its macroscopic structure and U-shaped disposition, could be classified as fundic type, as reported by de Moraes et al. (1997). In teleosts, the diversity of stomach shapes and structures is vast, even absent in families such as Cyprinidae, Labridae, Gobiidae, Scaridae, Cyprinodontidae, and some members of Poeciliidae (Genten et al., 2008; Ray & Ringø, 2014). The celomic organization of *P. magdalenae* concerning the coilings and folds of the gastrointestinal tract (esophagus, stomach, and intestine) shows a significant similarity to what has been reported for Characids (Alonso et al., 2015). Similarly, for species like *Astyanax endy*, *Astyanax rutilus*, *Cheirodon interruptus*, *Aphyocharax anisitsi, Gymnocorymbus ternetzi, Bryconamericus thomasi, Markiana nigripinnis, Characidium borellii*; also belonging to the characid group (Alonso et al., 2015). The same report also identified histological organization patterns for the entire stomach structure very similar to what was observed in this study for *P. magdalenae*, including structural peculiarities regarding the thickness of the muscular layer of the pyloric stomach (Fig. 6A) and coinciding with what has been reported for *P. lineatus* (Bowen, 2022).

The muscular tissue that lines the pyloric stomach region serves functions similar to a gizzard in herbivorous and detritivorous fish, aiding in the degradation of plant fibers and other non-living organic matter through the mechanical action of muscular movements (Burns, 2021; de Moraes et al., 1997). In the case of *P. lineatus*, the retention of mineral particles (sand) during the digestive process in the pyloric region has been confirmed, facilitating the grinding process of detritus ingested by the fish. This retention and selection of particles suggest muscular control that allows for the retention and mixing of food during this process, followed by its subsequent movement towards the intestine (Bowen, 2022; Guzmán-Beltran et al., 2013; Ramírez-Espitia et al., 2020). Despite the similarities between the gastrointestinal tracts of *P. magdalenae* and *P. lineatus*, studies are needed to provide precise information on how mechanical, enzymatic, and nutrient assimilation processes occur in *P. magdalenae.*

The mucins detected in the different regions of *P. magdalenae*'s stomach in this study exclusively corresponded to neutral mucins (Fig. 5, Fig. 6, and Table 3), as similarly observed in other species such as *B. amazonicus* (Vidal et al., 2020), *Hyphessobrycon anisitsi,* and *G. ternetzi* (Leiv-Leknes, 2011; Leiv-Leknes, 2015). Neutral mucins play a significant role in protecting the intestinal mucosa against mechanical damage from elements that are difficult to digest, as is the case with herbivores and detritivores, while also shielding the tissue from damage caused by hydrochloric acid secreted by oxynticopeptic cells and the enzymatic activity of the stomach (Domeneghini et al., 2005; Genten et al., 2008). In this study, the presence of this cell type was confirmed in the cardiac region of *P. magdalenae*'s stomach.

With TEM, the epithelial tissue in the pyloric region of *P. magdalenae*'s stomach confirmed the structure of simple columnar epithelium devoid of acinar glands and a marked granularity corresponding to the neutral mucins detected with Periodic Acid-Schiff (PAS) staining. This morphology is consistent with species from the characid group, including *A. endy, A. rutilus, C. interruptus, A. anisitsi, G. ternetzi, B. thomasi, M. nigripinnis*, and *C. borellii* (Alonso et al., 2015). While the aforementioned study analyzed morphology using optical microscopy, it provides a detailed description that aligns with the findings revealed by TEM in this study. Studies in other taxonomic groups, such as Siluriformes, including the species *C. gariepinus* (Moawad et al., 2016), Salmoniformes with *Brachymystax tsinlingensis* (Xiong et al., 2019) and Perciriformes with *Perca fluviatilis* (Noaillac-Depeyre & Gas, 1982), report intracellular granularities related to the secretory activity of the tissue in the stomach epithelium observed with TEM. At the intraepithelial level, electron-dense cells with large nuclei and little cytoplasm corresponding to migratory lymphocytes were also observed, similar to what was seen with TEM in Pimelodus pictus (Olaya et al., 2007) y *Lates niloticus* (Namulawa et al., 2015).

The intestinal morphology of *P. magdalenae*, particularly its length and coiling within the coelomic cavity, is consistent with findings reported for various species of detritivorous fish across different taxonomic groups and remains consistent within the *Prochilodontidae* genus (Bowen, 2022; Burns, 2021; Nachi et al., 1998). The intestinal length, typically ranging between 3 and 6 times the body length, the abundant number of pyloric caeca (which in this study correspond to the anterior portion of the intestine), numerous epithelial folds, and the increased volume of the coelomic cavity, represent convergent evolutionary adaptations in detritivorous fish. A larger surface area is necessary for efficient nutrient absorption in a diet low in nutrients (Burns, 2021; Nachi et al., 1998).

The observations conducted on the ultrastructure of the intestinal epithelium of *P. magdalenae* revealed elongated enterocytes in the epithelial composition, with oval nuclei exhibiting notable electrodensity towards the basal part of the cell. Towards the apical region, the cell membrane is adorned with numerous microvilli, accompanied by a cytoplasm of higher electrodensity compared to the basal zone of the enterocyte. Microvilli represent a characteristic feature of the intestinal epithelium in fish, augmenting the surface area of the cell membrane projected towards the intestinal lumen (Alonso et al., 2015; Bosi et al., 2022; Namulawa et al., 2015). This morphology maintains a close association with the physiological traits observed in detritivorous fish species. Similarly, the presence of some goblet cells producing mixed mucins is observed. Histologically, the study of the intestine of *P. magdalenae* revealed the presence of neutral and sulfated acidic mucins, as well as carboxylated mucins, with moderate intensity in the anterior and middle segments due to the low number of mucus-producing cells in these segments.

In the anterior portion, a slight predominance of neutral mucins over acidic mucins was observed. In the middle and posterior segments, there was a predominance of acidic mucins, mainly carboxylated. The presence of both acidic and neutral mucins in the intestine has been widely reported across various species and taxonomic groups. Some studies have yielded similar results for *T. sparrmanii* (Okuthe & Bhomela, 2021), *O. niloticus* (Phrompanya et al., 2019), and *B. amazonicus* (Vidal et al., 2020). The presence of different types of mucins in the analyzed intestinal regions in this study is associated with the absorption of proteins, ions, and other particles throughout the intestine (Bosi et al., 2022). These mucins also play a role in protecting the epithelium against pathogens and facilitating food and fecal transit by lubricating the walls of the intestinal lumen (Genten et al., 2008). Understanding the distribution of acidic and neutral mucins in the gastrointestinal tract of *P. magdalenae* is crucial for establishing frameworks regarding fish welfare, as observed in parasitic infestations by *Enteromyxum leei* in *S. aurata*. Additionally, knowledge of the gastrointestinal tract's ultrastructure would enable the detection of potential damage caused by microorganisms to the microvilli in different gastrointestinal tract regions (Ringø et al., 2007), as well as determining fish exposure to various contaminants such as herbicides or heavy metals (Samanta et al., 2018).

The study reveals distinct morphological and histochemical characteristics of *P. magdalenae*'s gastrointestinal tract. The stomach exhibits a U-shaped, fundic-type structure with three regions (cardiac, fundic, and pyloric), containing exclusively neutral mucins for mucosal protection. A notable thick muscular layer in the pyloric region aids in mechanical food degradation. The intestine shows considerable length (intestinal coefficient 2.87 ± 0.19), with multiple organized loops occupying the celomic cavity. Mucin distribution varies along the tract: the anterior intestine shows predominantly neutral mucins, while middle and posterior segments contain mainly carboxylated acidic mucins associated with nutrient absorption. The Zihler index (1.33 ± 0.10) and morphological features confirm its herbivorous/detritivorous feeding habits. These findings provide baseline data for the conservation and commercial production of this vulnerable endemic species.

**Ethical statement:** The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

## ACKNOWLEDGMENTS

This work was supported by ISAGEN S.A., Piscícola San Silvestre S.A., HISTOLAB, and Universidad de Antioquia within the framework of the agreement 33/121 and 33/02168, as a part of the Management Program for protecting fish and fishing resources of the Sogamoso River and its floodplain. The funder did not determine the study design, collection, analysis, data interpretation, or the decision to submit it for publication. Except for A.L.E-P, all authors declare no other competing interests.

## REFERENCES

Abdel-Tawwab, M., Hagras, A. E., Elbaghdady, H. A. M., & Monier, M. N. (2015). Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): Growth performance, whole-body composition, and innate immunity. *Aquaculture International, 23*(5), 1261–1274. https://doi.org/10.1007/s10499-015-9882-y

Abumandour, M. M. A., & El-Bakary, N. E. R. (2018). Morphological descriptions of the esophagus of the sea bream (*Sparus aurata*, Linneaus 1758). *Russian Journal of Marine Biology, 44*(2), 135–140. https://doi.org/10.1134/S1063074018020025

Agbugui, M. O., & Oniye, S. J. (2013). Some morphometric parameters of *Pomadasys Jubelini* in the New Calabar–Bonny River, Porthacourt, Nigeria. *Academia Arena, 5*(8), 1–4.

Agostinho, A. A., Pelicice, F. M., & Gomes, L. C. (2008). Dams and the fish fauna of the Neotropical region: Impacts and management related to diversity and fisheries. *Brazilian Journal of Biology, 68*(Suppl. 4), 1119–1132. https://doi.org/10.1590/S1519-69842008000500019

Albus, U. (2012). [Review of the book *Guide for the care and use of laboratory animals* (8th ed.), by National Research Council]. *Laboratory Animals, 46*(4), 373–374. https://doi.org/10.1258/la.2012.150312

Allan, J. D., & Castillo, M. M. (2007). Human impacts. In *Stream Ecology: Structure and function of running waters* (pp. 317–357). Springer Netherlands. https://doi.org/10.1007/978-1-4020-5583-6_13

Alonso, F., Mirande, J. M., & Pandolfi, M. (2015). Gross anatomy and histology of the alimentary system of Characidae (Teleostei: Ostariophysi: Characiformes) and potential phylogenetic information. *Neotropical Ichthyology, 13*(2), 273–286. https://doi.org/10.1590/1982-0224-20140137

Atencio-García, V., Hernández-Muñoz, J., & Pardo-Carrasco, S. (2008). Descripción morfológica del tubo digestivo de juveniles de rubio *Salminus affinis* (Pisces: Characidae). *Acta Biológica Colombiana, 13*(3), 97–110.

Awaad, A. S., Moawad, U. K., & Tawfiek, M. G. (2014). Comparative histomorphological and histochemical studies on the Oesophagus of Nile tilapia *Oreochromis niloticus* and African catfish *Clarias gariepinus*. *Journal of Histology, 2014*(1), e987041. https://doi.org/10.1155/2014/987041

Baldisserotto, B., Urbinati, E. C., & Cyrino, J. E. P. (Eds.). (2019). *Biology and physiology of freshwater neotropical fish*. Academic Press. https://doi.org/10.1016/C2017-0-03766-7

Benbow, M. E., Receveur, J. P., & Lamberti, G. A. (2020). Death and decomposition in aquatic ecosystems. *Frontiers in Ecology and Evolution, 8*(17), 1–12. https://doi.org/10.3389/fevo.2020.00017

Benedito, E., Santana, A., & Werth, M. (2018). Divergence in energy sources for *Prochilodus lineatus* (Characiformes: Prochilodontidae) in Neotropical floodplains. *Neotropical Ichthyology, 16*(4), e160130. https://doi.org/10.1590/1982-0224-20160130

Bosi, G., Merella, P., Maynard, B. J., & Sayyaf-Dezfuli, B. (2022). Microscopic characterization of the mucous cells and their mucin secretions in the alimentary canal of the Blackmouth catshark *Galeus melastomus* (Chondrichthyes: Elasmobranchii). *Fishes, 7*(1), 8. https://doi.org/10.3390/fishes7010008

Bowen, S. H. (2022). Digestion and assimilation of benthic biofilm by the sábalo, *Prochilodus lineatus*. *Journal of Fish Biology, 100*(1), 107–116. https://doi.org/10.1111/jfb.14924

Bowen, S. H., Gu, B., & Huang, Z. (2006). Diet and digestion in Chinese mud carp *Cirrhinus molitorella* compared with other Ilyophagous fishes. *Transactions of the American Fisheries Society, 135*(5), 1383–1388. https://doi.org/10.1577/T05-158.1

Burns, M. D. (2021). Adaptation to herbivory and detritivory drives the convergent evolution of large abdominal cavities in a diverse freshwater fish radiation (Otophysi: Characiformes). *Evolution, 75*(3), 688–705. https://doi.org/10.1111/evo.14178

Caballero, M. J., Izquierdo, M. S., Kjørsvik, E., Fernández, A. J., & Rosenlund, G. (2004). Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short- or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. *Journal of Fish Diseases, 27*(9), 531–541. https://doi.org/10.1111/j.1365-2761.2004.00572.x

Cabrera-Páez, Y., Aguilar-Betancourt, C., & González-Sansón, G. (2008). Indicadores morfológicos y reproductivos del pez *Gambusia puncticulata* (Poeciliidae) en sitios muy contaminados del río Almendares, Cuba. *Revista de Biología Tropical, 56*(4), 1991–2004. https://doi.org/10.15517/rbt.v56i4.5774

Cañas-Alva, C. M., Moya-Vásquez, L. C., Vargas-Rojas, M., & Mercado-Torres, A. (2020). Relación longitud-peso y factor de condición de *Prochilodus nigricans* y *Potamorhina altamazonica* en la cuenca del río Tahuayo, Loreto (Perú). *Folia Amazónica, 29*(1), 37–50. https://doi.org/10.24841/fa.v29i1.502

Cao, X. J., & Wang, W. M. (2009). Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. *Anatomia, Histologia, Embryologia, 38*(4), 254–261. https://doi.org/10.1111/j.1439-0264.2009.00932.x

Castro, R. M. C., & Vari, R. P. (2004). *Detritivores of the South American fish family Prochilodontidae (Teleostei:*

*Ostariophysi: Characiformes): A phylogenetic and revisionary study.* (No. 622). Smithsonian Contributions and Studies Series. https://doi.org/10.5479/si.00810282.622

Cifuentes, R., González, J., Montoya, G., Jara, A., Ortíz, N., Piedra, P., & Habit, E. (2012). Weight-length relationships and condition factor of native fish from San Pedro River (Valdivia River basin, Chile). *Gayana (Concepción), 76*(Suppl. 1), 86–100. https://doi.org/10.4067/S0717-65382012000100009

Contreras-Almazo, I. A. E., Cantillo-González, O. E., Coronel-Ortiz, I. Y., González-Rentería, M., & Pacheco-Peñaranda, E. (2019). Aspectos biológicos y reproductivos en hembras *Prochilodus magdalen*ae (Steindachner, 1879) Ciénagas del Departamento del Cesar, Colombia. *AquaTIC: Revista Electrónica de Acuicultura,* (54), 1–14.

Cornick, S., Tawiah, A., & Chadee, K. (2015). Roles and regulation of the mucus barrier in the gut. *Tissue Barriers, 3*(1–2), e982426. https://doi.org/10.4161/21688370.2014.982426

Couto, T. B. A., Messager, M. L., & Olden, J. D. (2021). Safeguarding migratory fish via strategic planning of future small hydropower in Brazil. *Nature Sustainability, 4*(5), 409–416. https://doi.org/10.1038/s41893-020-00665-4

Cyrino, J. E. P., Bureau, D., & Kapoor, B. G. (Eds.). (2008). *Feeding and digestive functions in fishes*. Taylor & Francis Group.

Day, R. D., Tibbetts, I. R., & Secor, S. M. (2014). Physiological responses to short-term fasting among herbivorous, omnivorous, and carnivorous fishes. *Journal of Comparative Physiology B, 184*(4), 497–512. https://doi.org/10.1007/s00360-014-0813-4

de Moraes, M. F. P. G., Barbola, I. F., & Guedes, É. A. C. (1997). Alimentação e relações morfológicas com o aparelho digestivo do "curimbatá", *Prochilodus lineatus* (Valenciennes) (Osteichthyes, Prochilodontidae), de uma lagoa do Sul do Brasil. *Revista Brasileira de Zoologia, 14*(1), 169–180. https://doi.org/10.1590/S0101-81751997000100015

Díaz, A. O., García, A. M., & Goldemberg, A. L. (2008). Glycoconjugates in the mucosa of the digestive tract of *Cynoscion guatucupa*: A histochemical study. *Acta Histochemica, 110*(1), 76–85. https://doi.org/10.1016/j.acthis.2007.08.002

Domeneghini, C., Arrighi, S., Radaelli, G., Bosi, G., & Veggetti, A. (2005). Histochemical analysis of glycoconjugate secretion in the alimentary canal of *Anguilla anguilla* L. *Acta Histochemica, 106*(6), 477–487. https://doi.org/10.1016/j.acthis.2004.07.007

Doria-González, M. A., Espitia-Galvis, A. M., Segura-Guevara, F. F., & Olaya-Nieto, C. W. (2020). Biología reproductiva del bocachico *Prochilodus magdalenae* (Prochilodontidae) en el río San Jorge, Colombia. *Acta Biológica Colombiana, 26*(1), 54–61. https://doi.org/10.15446/abc.v26n1.82907

dos Santos, M. L., Arantes, F. P., Santiago, K. B., & dos Santos, J. E. (2015). Morphological characteristics of the digestive tract of *Schizodon knerii* (Steindachner, 1875), (Characiformes: Anostomidae): An anatomical, histological and histochemical study. *Anais da Academia Brasileira de Ciências, 87*(2), 867–878. https://doi.org/10.1590/0001-3765201520140230

Favero, G. C., Costa dos Santos, F. A., Soares da Costa-Júlio, G. S., Soares-Batista, F., Teixeira-Bonifácio, C., Araújo-Torres, I. F., Oliveora-Paranhos, C., & Kennedy-Luz, R. (2022). Effects of water temperature and feeding time on growth performance and physiological parameters of *Piaractus brachypomus* juveniles. *Aquaculture, 548*(Part 2), 737716. https://doi.org/10.1016/j.aquaculture.2021.737716

Figueiredo-Silva, A. C., Saravanan, S., Schrama, J. W., Panserat, S., Kaushik, S., & Geurden, I. (2013). A comparative study of the metabolic response in rainbow trout and Nile tilapia to changes in dietary macronutrient composition. *British Journal of Nutrition, 109*(5), 816–826. https://doi.org/10.1017/S000711451200205X

Firmino, J. P., Vallejos-Vidal, E., Sarasquete, C., Ortiz-Delgado, J. B., Balasch, J. C., Tort, L., Estevez, A., Reyes-López, F. E., & Gisbert, E. (2020). Unveiling the effect of dietary essential oils supplementation in *Sparus aurata gills* and its efficiency against the infestation by *Sparicotyle chrysophrii*. *Scientific Reports, 10*(1), 17764. https://doi.org/10.1038/s41598-020-74625-5

Flecker, A. S. (1996). Ecosystem engineering by a dominant detritivore in a diverse tropical stream. *Ecology, 77*(6), 1845–1854. https://doi.org/10.2307/2265788

Genten, F., Terwinghe, E., & Danguy, A. (Eds.). (2008). *Atlas of Fish Histology*. CRC Press. https://doi.org/10.1201/9780367803599

Graham, L., & Orenstein, J. M. (2007). Processing tissue and cells for transmission electron microscopy in diagnostic pathology and research. *Nature Protocols, 2*(10), 2439–2450. https://doi.org/10.1038/nprot.2007.304

Guzmán-Beltran, L., Santana, V. D., Verdugo, M. H., Gómez-Ramirez, E., & Hurtado-Giraldo, H. (2013). Descripción anatómica e histológica del tracto digestivo de Nicuro *Pimelodus blochii* (Valenciennes, 1840). *Orinoquia, 17*(1), 102–110.

Hasim, Tuheteru, J., & Fazrin, D. N. (2021). Comparison of growth pattern, condition factor, gonadosomatic index of *Glosogobius giuris* and *Ophieleotris aporos* in Limboto Lake, Gorontalo, Indonesia. *Biodiversitas: Journal of Biological Diversity, 22*(6), 3388–3393. https://doi.org/10.13057/biodiv/d220646

Hedayati, S. A., Bagheri, T., Hoseinifar, S. H., & Van Doan, H. (2020). Growth performances and hemato-immunological responses of common carp (*Cyprinus carpio* Linnaeus, 1758) to fermented *Aspergillus oryzae*. *Iranian Journal of Fisheries Sciences, 19*(4), 1749–1756. Scopus. https://doi.org/10.22092/ijfs.2018.117403

Jiao, F., Zhang, L., Limbu, S. M., Yin, H., Xie, Y., Yang, Z., Shang, Z., Kong, L., & Rong, H. (2023). A comparison of digestive strategies for fishes with different feeding habits: Digestive enzyme activities, intestinal morphology, and gut microbiota. *Ecology and Evolution, 13*(9), e10499. https://doi.org/10.1002/ece3.10499

Karachle, P., & Stergiou, K. I. (2010). Intestine morphometrics of fishes: A compilation and analysis of bibliographic data. *Acta Ichthyologica Et Piscatoria, 40*(1), 45–54. https://doi.org/10.3750/AIP2010.40.1.06

Knutsen, H. R., Sørensen, S. R., Munk, P., Bardal, T., & Kjørsvik, E. (2021). Digestive tract and the muscular pharynx/esophagus in wild leptocephalus larvae of European eel (*Anguilla anguilla*). *Frontiers in Marine Science, 8*. https://doi.org/10.3389/fmars.2021.545217

Koshio, S. (2015). Immunotherapies targeting fish mucosal immunity – Current knowledge and future perspectives. *Frontiers in Immunology, 6*, 643. https://doi.org/10.3389/fimmu.2015.00643

Kroon, F., Streten, C., & Harries, S. (2017). A protocol for identifying suitable biomarkers to assess fish health: A systematic review. *PloS One, 12*(4), e0174762–e0174762. https://doi.org/10.1371/journal.pone.0174762

Kumar, G. L., & Kiernan, J. A. (Eds.). (2010). *Education Guide - Special Stains and H & E: Pathology.* Dako.

Lasso, C. A., Agudelo-Córdoba, E., Jiménez-Segura, L. F., Ramírez-Gil, H., Morales-Betancourt, M., Ajiaco-Martínez, R. E., de Paula Gutiérrez, F., Usma-Oviedo, J. S., Muñoz-Torres, S. E., & Sanabria-Ochoa, A. I. (Eds.). (2011). *I. Catálogo de los recursos pesqueros continentales de Colombia*. Serie Editorial Recursos Hidrobiológicos y Pesqueros Continentales de Colombia. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH).

Leiv-Leknes, I. (2011). Histochemical studies on mucin-rich cells in the digestive tract of a teleost, the Buenos Aires tetra (*Hyphessobrycon anisitsi*). *Acta Histochemica, 113*(3), 353–357. https://doi.org/10.1016/j.acthis.2010.01.010

Leiv-Leknes, I. (2015). Mucin in epithelial cells in oesophagus and stomach of black tetra, *Gymnocorymbus ternetzi* (Characidae, Teleostei). *Zoomorphology, 134*(2), 269–277. https://doi.org/10.1007/s00435-015-0256-9

Liu, Z., Chang, H., Xu, F., Zhao, H., Zhu, L., Sun, Z., Yang, M., Wang, X., & Ma, A. (2023). Genome-wide association study of feed conversion ratio in turbot (*Scophthalmus maximus*) based on genome resequencing. *Aquaculture Reports, 33*, 101804. https://doi.org/10.1016/j.aqrep.2023.101804

Mancera-Rodríguez, N. J., Castellanos-Barliza, J., & Urrego-Ballestas, D. (2016). Reproducción de *Saccodon dariensis* (Teleostei: Parodontidae) en afluentes del río Guatapé, cuenca del río Magdalena, Colombia. *Revista de Biología Tropical, 64*(2), 635–653. https://doi.org/10.15517/rbt.v64i2.20691

Marchetti, L., Capacchietti, M., Sabbieti, M. G., Accili, D., Materazzi, G., & Menghi, G. (2006). Histology and carbohydrate histochemistry of the alimentary canal in the rainbow trout *Oncorhynchus mykiss*. *Journal of Fish Biology, 68*(6), 1808–1821. https://doi.org/10.1111/j.0022-1112.2006.01063.x

Moawad, U., Awaad, A., & Tawfiek, M. (2016). Histomorphological, histochemical and ultrastructural studies on the stomach of the adult African catfish (*Clarias gariepinus*). *Journal of Microscopy and Ultrastructure, 5*(3), 155–166.

Mojica, J. I., Usma, J. S., Álvarez-León, R., & Lasso, C. A. (Eds.). (2012). *Libro Rojo de Peces Dulceacuícolas de Colombia*. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt.

Morales-González, G. (2023). *Morfo-ecología alimenticia de machos del género de peces vivíparos* Poeciliopsis *(Cyprinodontiformes: Poeciliidae)* [Tesis de licenciatura, Universidad Autónoma Metropolitana]. XOOK. https://repositorio.xoc.uam.mx/jspui/handle/123456789/43583

Moreno, J. M., Aguilar, F. A., Boada, N. S., Rojas, J. A., & Prieto, C. (2019). Análisis morfométrico e índices corporales del capitán de la sabana. *Revista de la Facultad de Medicina Veterinaria y de Zootecnia, 66*(2), 141–153. https://doi.org/10.15446/rfmvz.v66n2.82433

Mosquera-Ramos, D. F., & Gómez-Ramírez, E. (2024). Anatomía macro y microscópica del sistema digestivo del dentón (*Megaleporinus muyscorum*) en etapa adulta. *Acta Biológica Colombiana, 29*(1), 61–69. https://doi.org/10.15446/abc.v29n1.100063

Mozsár, A., Boros, G., Sály, P., Antal, L., & Nagy, S. A. (2015). Relationship between Fulton's condition factor and proximate body composition in three freshwater fish species. *Journal of Applied Ichthyology, 31*(2), 315–320. https://doi.org/10.1111/jai.12658

Nachi, A. M., Hernandez-Blazquez, F. J., Barbieri, R. L., Leite, R. G., Ferri, S., & Phan, M. T. (1998). Intestinal histology of a detritivorous (iliophagous) fish *Prochilodus scrofa* (Characiformes, Prochilodontidae). *Annales Des Sciences Naturelles - Zoologie et Biologie Animale, 19*(2), 81–88. https://doi.org/10.1016/S0003-4339(98)80002-6

Namulawa, V. T., Kato, C. D., Nyatia, E., Rutaisire, J., & Britz, P. J. (2015). Transmission electron microscopy of the gastrointestinal tract of nile perch *Lates niloticus*. *International Journal of Morphology, 33*(2), 751–758. https://doi.org/10.4067/S0717-95022015000200053

Natale, S., Bertini, A., Gisbert, E., Andree, K. B., Benini, E., Vallainc, D., Gatta, P. P., Bonaldo, A., & Parma, L. (2025). First insight into temporal variation of digestive enzyme activities in flathead grey mullet *(Mugil cephalus*) during the ongrowing phase. *Aquaculture Reports, 41,* 102652. https://doi.org/10.1016/j.aqrep.2025.102652

Noaillac-Depeyre, J., & Gas, N. (1982). Ultrastructure of endocrine cells in the stomach of two teleost fish, *Perca fluviatilis* L. and *Ameiurus nebulosus* L. *Cell and Tissue Research, 221*(3), 657–678. https://doi.org/10.1007/BF00215709

Nunes, C., Silva, A., Soares, E., & Ganias, K. (2011). The use of hepatic and somatic indices and histological information to characterize the reproductive dynamics of Atlantic sardine *Sardina pilchardus* from the Portuguese Coast. *Marine and Coastal Fisheries, 3*(1), 127–144. https://doi.org/10.1080/19425120.2011.556911

Okuthe, G. E., & Bhomela, B. (2021). Morphology, histology and histochemistry of the digestive tract of the banded tilapia, *Tilapia sparrmanii* (Perciformes: Cichlidae). *Zoologia (Curitiba), 37*, e51043. https://doi.org/10.3897/zoologia.37.e51043

Olaya, C. M., Ovalle, C. H., Gómez Ramírez, E., Rodríguez Caicedo, D., Caldas Martínez, M. L., & Hurtado Giraldo, H. (2007). Histología y morfometría del sistema digestivo del silúrido bagre tigrito (*Pimelodus pictus*). *Revista de la Facultad de Medicina Veterinaria y de Zootecnia, 54*, 311–323. https://repositorio.unal.edu.co/handle/unal/24915

Ortiz-Ruiz, M., López-Flórez, C., Castro-Rebolledo, M. I., Baldisserotto, B., & Gómez-Ramírez, E. (2024). Anatomy, histology and ultrastructure of the digestive tract in Andean fish (*Trichomycterus bogotensis*) and ecological implications. *Zoomorphology, 143*(2), 433–441. https://doi.org/10.1007/s00435-023-00634-3

Osorio-Urtecho, K. (2018). *Efecto de la salinidad sobre el metabolismo del glucógeno hepático y cerebral tras la ingesta de alimento, usando como organismo de ensayo a Tilapia Roja* (Oreochromis spp.) [Tesis de maestría]. Universidad Nacional Autónoma de Nicaragua.

Oviedo-Montiel, H. de J. (2021). *Cladóceros en el desarrollo del sistema digestivo de larvas de blanquillo* Sorubim cuspicaudus [Tesis de maestría, Universidad de Córdoba]. Repositorio institucional: Universidad de Córdoba. https://repositorio.unicordoba.edu.co/handle/ucordoba/4284

Palladino, A., de Felice, E., Attanasio, C., Barone, C. M. A., Crasto, A., D'Angelo, L., Giaquinto, D., Lambiase, C., Scocco, P., Serrapica, F., & Maruccio, L. (2023). A morphological and ultrastructural study of the anterior digestive tract of adult Nile tilapia *Oreochromis niloticus*. *Animals, 13*(3), 420. https://doi.org/10.3390/ani13030420

Pereira, B. F., da Silva-Alves, R. M., Alves, A. L., Senhorini, J. A., de Alcântara-Rocha, R. de C. G., Hakime-Scalize, P., Pitol, D. L., & Caetano, F. H. (2014). Effects of biodegradable detergents in morphological parameters of liver in two neotropical fish species (*Prochilodus lineatus* and *Astyanax altiparanae*). *Microscopy Research, 2*(2), 39–49. https://doi.org/10.4236/mr.2014.22006

Phrompanya, P., Saenphet, K., & Saenphet, S. (2019). Comparative histochemical study of the gastrointestinal tracts of the Nile tilapia (*Oreochromis niloticus*) and the hybrid catfish (*Clarias batrachus* x *Clarias gariepinus*). *Acta Histochemica, 121*(3), 261–267. https://doi.org/10.1016/j.acthis.2019.01.003

Radkhah, A., & Eagderi, S. (2015). Length-weight and length-length relationships and condition factor of six cyprinid fish species of Zarrineh River (Urmia Lake basin, Iran). *Iranian Journal of Ichthyology, 2*(1), 61–64. https://doi.org/10.22034/iji.v2i1.12

Ragheb, E. (2023). Length-weight relationship and well-being factors of 33 fish species caught by gillnets from the Egyptian Mediterranean waters off Alexandria. *Egyptian Journal of Aquatic Research, 49*(3), 361–367. https://doi.org/10.1016/j.ejar.2023.01.001

Ramírez-Espitia, E. J., Hurtado-Giraldo, H., & Gómez-Ramírez, E., (2020). Anatomía general, histología y morfometría del sistema digestivo del pez *Pterophyllum scalare* (Perciformes: Cichlidae). *Revista de Biología Tropical, 68*(4), 1371–1383. https://doi.org/10.15517/rbt.v68i4.40393

Ray, A. K., & Ringø, E. (2014). The gastrointestinal tract of fish. In D. Merrifield, & E. Ringø (Eds.), *Aquaculture nutrition: Gut health, probiotics and prebiotics* (pp. 1–13). John Wiley & Sons. https://doi.org/10.1002/9781118897263.ch1

Reis, R. E., Albert, J. S., Di Dario, F., Mincarone, M. M., Petry, P., & Rocha, L. A. (2016). Fish biodiversity and conservation in South America. *Journal of Fish Biology, 89*(1), 12–47. https://doi.org/10.1111/jfb.13016

Ringø, E., Myklebust, R., Mayhew, T. M., & Olsen, R. E. (2007). Bacterial translocation and pathogenesis in the digestive tract of larvae and fry. *Aquaculture, 268*(1–4), 251–264. https://doi.org/10.1016/j.aquaculture.2007.04.047

Rodrigues, A. P. O., & Cargnin-Ferreira, E. (2017). Morphology and histology of the pirarucu (*Arapaima gigas*) digestive tract. *International Journal of Morphology, 35*(3), 950–957. https://doi.org/10.4067/S0717-95022017000300025

Salem, M., Al-Tobasei, R., Ali, A., Lourenco, D., Gao, G., Palti, Y., Kenney, B., & Leeds, T. D. (2018).

Genome-wide association analysis with a 50k transcribed gene snp-chip identifies QTL affecting muscle yield in rainbow trout. *Frontiers in Genetics, 9*, 387. https://doi.org/10.3389/fgene.2018.00387

Salinas-Torres, D. (2011). *El sistema digestivo de peces óseos* [Tesis de maestría, Universidad Nacional Autónoma de México]. TESIUNAM. https://hdl.handle.net/20.500.14330/TES01000672144

Samanta, P., Kumari, P., Pal, S., Mukherjee, A. K., & Ghosh, A. R. (2018). Histopathological and ultrastructural alterations in some organs of *Oreochromis niloticus* exposed to glyphosate-based herbicide, Excel Mera 71. *Journal of Microscopy and Ultrastructure, 6*(1), 35–43. https://doi.org/10.1016/j.jmau.2017.03.004

Serrano-López, J. N., Soto-López, K., Ochoa-Báez, R. I., O'Sullivan, J., & Galván-Magaña, F. (2021). Morphometry and histology to assess the maturity stage of three endangered devil ray species (Elasmobranchii: Mobulidae) from the Gulf of California. *Aquatic Conservation: Marine and Freshwater Ecosystems, 31*(7), 1624–1635. https://doi.org/10.1002/aqc.3548

Vega-Contreras, N. A., Galvis, F., & Salazar-Mercado, S. A. (2017). Relaciones evolutivas de los peces *Prochilodus reticulatus* y *Prochilodus magdalenae* (Characiformes: Prochilodontidae). *Revista de Ciencias, 21*(1), 161–171. https://doi.org/10.25100/rc.v21i1.6348

Verma, C. R., Gorule, P. A., Kumkar, P., Kharat, S. S., & Gosavi, S. M. (2020). Morpho-histochemical adaptations of the digestive tract in Gangetic mud-eel *Ophichthys cuchia* (Hamilton 1822) support utilization of mud-dwelling prey. *Acta Histochemica, 122*(7), 151602. https://doi.org/10.1016/j.acthis.2020.151602

Vidal, M. R., Ruiz, T. F. R., dos Santos, D. D., Gardinal, M. V. B., de Jesus, F. L., Faccioli, C. K., Vicentini, I. B. F., & Vicentini, C. A. (2020). Morphological and histochemical characterisation of the mucosa of the digestive tract in matrinxã *Brycon amazonicus* (Teleostei: Characiformes). *Journal of Fish Biology, 96*(1), 251–260. https://doi.org/10.1111/jfb.14217

Wilson, J. M., & Castro, L. F. C. (2010). Morphological diversity of the gastrointestinal tract in fishes. In M. Grosell, A. P. Farrell, & C. J. Brauner (Eds.), *Fish physiology* (Vol. 30, pp. 1–55). Academic Press. https://doi.org/10.1016/S1546-5098(10)03001-3

WWF Colombia & Autoridad Nacional de Acuicultura y Pesca (AUNAP). (2020). *La pesca en Colombia: del agua a la mesa.* Agenda del Mar. https://wwflac.awsassets.panda.org/downloads/libro_pesca_adm_2020baja_1.pdf

Xiong, D., Meng, Y., Yu, H., Liu, X., & Liu, H. (2019). Structure and ultrastructure studies of the digestive tract of the endangered Qinling lenok (*Brachymystax tsinlingensis* Li, 1966). *Iranian Journal of Fisheries Science, 18*(4), 981–995. https://dor.isc.ac/dor/20.1001.1.15622916.2019.18.4.16.7

Zaldúa, N., & Naya, D. E. (2014). Digestive flexibility during fasting in fish: A review. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 169*, 7–14. https://doi.org/10.1016/j.cbpa.2013.12.006