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Microscopic Characterization of the Mucous Cells and Their Mucin Secretions in the Alimentary Canal of the Blackmouth Catshark *Galeus melastomus* (Chondrichthyes: Elasmobranchii)

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Abstract: Sharks belong to the most primitive group of jawed vertebrates and have some special structural and functional features such as a cartilaginous skeleton, a spiral intestinal valve, and a rectal gland for osmoregulation. In January 2020, ten specimens of *Galeus melastomus*, the Blackmouth catshark, were collected from the Gulf of Asinara (North Sardinia, Italy) and the entire alimentary canal was studied using histochemical reactions to characterize the mucous cell types. In the alimentary canal of *G. melastomus*, mucous cells mainly secrete a mixture of acidic and neutral mucins. Of the acidic mucins, only the carboxylated type was present in mucous cells of the stomach, while the sulfated type predominated in the esophagus and the intestines. The use of lectins revealed a distribution of sugar residues in mucins related to cellular activities of the different regions of the catshark alimentary canal. The current study is the first report to characterize the intestinal mucous cells of *G. melastomus* and to provide quantitative data on their different populations in the alimentary canal.

Keywords: shark; alimentary canal; mucous cells; mucins histochemistry; lectin histochemistry

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1. Introduction

Sharks evolved about 400 million years ago, making them one of the oldest living jawed vertebrates [1,2]. Most sharks are predators that feed infrequently with a long time between meals [3,4]. The Blackmouth catshark (*Galeus melastomus*), a member of the family Scyliorhinidae, is widely distributed in the northeastern Atlantic Ocean and the western Mediterranean Sea, inhabiting the continental slope. It feeds mainly on bottom invertebrates including shrimps and cephalopods [5].

Sharks tend to swallow prey after little mastication, so the stomach stores and converts the prey tissue and/or organs to chyme. As in other predatory fish, their alimentary canal is short [6]. The stomach often is J-shaped, with a proximal descending and a distal ascending region [7]. The stomach is followed by the intestine. According to Hart et al. [1], the correct terms for the regions of shark intestines are proximal (not duodenum), spiral, and distal (not rectum). The most important region is the medially located spiral intestine with the spiral valve, which increases the absorptive surface area [7,8] and is also found in other elasmobranchs (skates, rays, and sharks), lampreys [9], and other primitive fishes such as sturgeon [10,11].

In the alimentary canal of vertebrates, the epithelial surface is protected by a mucus blanket made up of the mucins secreted by mucous cells. Mucins are high-molecular-weight, glycosylated proteins with a peptide backbone to which five main monosaccharides

bind [12,13]. The type of carbohydrate residues in a mucin molecule indicates the mucin's function either under normal or pathological conditions [14–17]. The carbohydrate composition of secreted mucins can be studied using the histochemical reaction with lectins, molecules synthesized in all living organisms with a high binding affinity to specific sugar residues [18].

In teleost fishes, the histochemistry and type of mucous cells of the alimentary canal have been well studied, but only a few papers have been published on the subject for cartilaginous fishes [7,8,19,20]. The lack of knowledge about the distribution of mucous cell types in the alimentary canal of elasmobranchs and the glycoconjugate composition of their secreted mucins prompted us to carry out this study. Our results are the first to provide: (1) direct evidence of the number of mucous cells of the *G. melastomus* alimentary canal; (2) information about the presence of different mucous cell types; (3) data on their different secretions related to the specific function of each region.

2. Materials and Methods

In January 2020, ten specimens of *Galeus melastomus* (6 males and 4 females) were collected in the Gulf of Asinara (Sardinia, western Mediterranean Sea) by commercial trawl fishing during a haul at 540–620 m depth. The mean total length (±standard deviation) of the ten catsharks was 45.2 ± 3.3 cm, and their mean total weight (±standard deviation) was 214.0 ± 48.1 g. Specimens were euthanized with an overdose of 125 mg/L of tricaine methanesulfonate (Sandoz, Basel, Switzerland) and immediately dissected onboard the ship. The entire alimentary canal of each catshark was removed and fixed in 10% neutral buffered formaldehyde solution. The fixative was also injected into the stomach and spiral intestine using a 10 mL syringe to allow better fixation of the gastrointestinal tract. Once in the laboratory, a piece (15 × 15 mm) was excised from each region of the alimentary canal of each specimen and fixed in 10% neutral buffered formaldehyde solution for a further 24 h. All fixed samples were then rinsed in several changes of 4 °C 70% ethanol before being stored in the same medium and sent to the University of Ferrara for embedding. After routine paraffin embedding, 5 µm-thick sections were obtained from each tissue block.

The mucin type secreted by mucous epithelial cells in each region of the alimentary canal was revealed with the Alcian blue 8GX (pH 2.5)/periodic acid Schiff (AB/PAS) sequence [21], the high iron diamines/Alcian blue 8GX (pH 2.5) (HID/AB) reactions [22,23], and lectin histochemistry. The AB/PAS stain differentiates among acidic (AB-positive, blue), neutral (PAS-positive, magenta), and mixed neutral+acidic (AB/PAS-positive, purple-violet) mucins. The HID/AB sequence distinguishes between acidic sulfated (HID-positive, brownish-black) and acidic carboxylated nonsulfated mucins (AB-positive, blue). The protocol for lectin histochemistry was previously described by Bosi et al. [16,24]. The six lectins employed, source codes, and primary sugar affinities are reported in Table 1. Replicates of each section were treated with each lectin plus 0.2 M of solution-associated sugars (Table 1) to inhibit lectin reactivity.

The stained sections were examined and photographed under an Olympus BX51 microscope (Olympus, Milan, Italy) equipped with a digital camera (Camedia C-5160, Olympus, 5.1 Mp) and image analysis software (DP-soft, version 3.2, Olympus, Milan, Italy). For quantitative studies, five fields from three sections of each of the six alimentary canal regions per fish (150 samples per region) at 400× magnification (40× Olympus lens, Numerical Aperture = 0.75) were photographed. Mucous cells containing different mucin types were distinguished and counted in the sections stained with AB/PAS and HID/AB reactions. The procedure was also used to count mucous cells positive for each lectin. Mucous cell counts were reported as mean number ± standard error per 100,000 µm^2 of epithelial section area.

Table 1. List of the biotinylated lectins used, their sources, and preferred binding carbohydrate specificities.

Name of Lectin	Acronym	Species Source: Latin Name (Common Name)	Vector Labs. Code	Major Sugar Specificity
Concanavalin-A	Con-A	*Canavalia ensiformis* (Jack bean)	B-1005	α-Mannose
Dolichos Biflorus Agglutinin	DBA	*Dolichos biflorus* (horse gram)	B-1035	N-acetyl-α-galactosamine
Peanut Agglutinin	PNA	*Arachis hypogaea* (peanut)	B-1075	Galactosyl(β-1,3)-N-acetyl-α-galactosamine
Sambucus Nigra Lectin	SNA	*Sambucus nigra* (Elderberry)	B-1305	Sialic acid-α-2,6-galactose
Ulex Europaeus Agglutinin I	UEA I	*Ulex europaeus* (gorse seed)	B-1065	Fucose-α-1,2-galactose
Wheat Germ Agglutinin	WGA	*Triticum vulgare* (wheat germ)	B-1025	N-acetyl-β-glucosamine

3. Results

Six main regions were distinguished in the alimentary canal of *G. melostomus*: esophagus, stomach, ascending part of the stomach, proximal intestine, spiral intestine with spiral valve, and distal intestine (Figure 1).

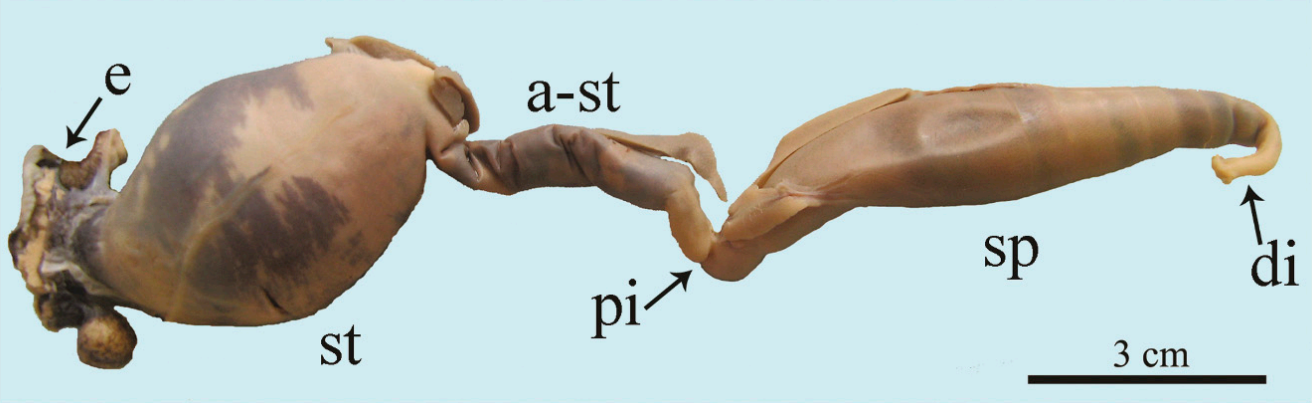

Figure 1. Photograph of the alimentary canal of *Galeus melastomus*. The entire digestive tract was removed from the fish to show the position of each region. e: esophagus; st: stomach; a-st: ascending part of the stomach; pi: proximal intestine; sp: spiral intestine with spiral valve; di: distal intestine.

The esophagus of the catshark contained many more mucous cells with mixed mucins than with either acidic or neutral mucins only (Table 2, Figure 2a). The epithelial brush border was Alcian blue-positive in the crypts of the esophageal folds (Figure 2a). Most of the acidic mucins were of the sulfated type (80.4%), with 19.6% of mucous cells containing carboxylated acidic mucins (Table 3, Figure 2b).

Five lectins tested, namely Dolichos Biflorus Agglutinin (DBA), Peanut Agglutinin (PNA), Sambucus Nigra Lectin (SNA), Ulex Europaeus Agglutinin I (UEA I), and Wheat Germ Agglutinin (WGA), gave a positive reaction in the mucous cells of the esophagus (Table 4). The reactivity to lectin WGA was observed in all the mucous cells (Figures 2c and 3). A low number of mucous cells was positive to the lectin SNA (Table 4, Figures 2d and 3). In the esophagus, the mucous cells reactive to the lectins PNA, DBA, and UEA I were 49.9%, 37.0%, and 21.2% of the total mucous cells, respectively (Table 4, Figure 3).

Table 2. Mean number of mucous cells (±standard error) per 100,000 μm^2 of epithelial area in the alimentary canal of Galeus melastomus containing acidic (Alcian blue-positive, AB), neutral (periodic acid Schiff-positive, PAS), and mixed (AB/PAS-positive) mucins. Mucous cells were counted in 5 fields (400× magnification), from 3 slides for each of the 10 Blackmouth catshark specimens (total of 150 microscopic fields).

Mucous Cell Types		Acidic	Neutral	Mixed (Acidic + Neutral)	Total
Gut Regions	Esophagus	46.8 ± 1.2 (24.0%)	52.7 ± 1.3 (27.1%)	95.2 ± 2.7 (48.9%)	193.6 ± 4.1 (100.0%)
	Stomach	0.0	120.7 ± 2.6 (36.3%)	211.6 ± 2.1 (63.7%)	332.9 ± 2.5 (100.0%)
	Ascending stomach	0.0	108.4 ± 2.1 (35.5%)	197.3 ± 1.8 (64.5%)	305.8 ± 4.8 (100.0%)
	Proximal intestine	35.9 ± 1.0 (29.1%)	38.7 ± 1.8 (31.4%)	48.6 ± 1.5 (39.4%)	123.2 ± 2.4 (100.0%)
	Spiral intestine	29.8 ± 0.8 (24.2%)	20.9 ± 0.8 (17.0%)	54.6 ± 1.8 (44.3%)	105.7 ± 3.3 (100.0%)
	Spiral valve	34.3 ± 0.9 (32.1%)	20.9 ± 0.7 (19.6%)	51.6 ± 1.2 (48.3%)	106.9 ± 2.3 (100.0%)
	Distal intestine	101.7 ± 1.9 (34.9%)	67.4 ± 2.0 (23.1%)	122.4 ± 3.0 (42.0%)	291.6 ± 5.7 (100.0%)

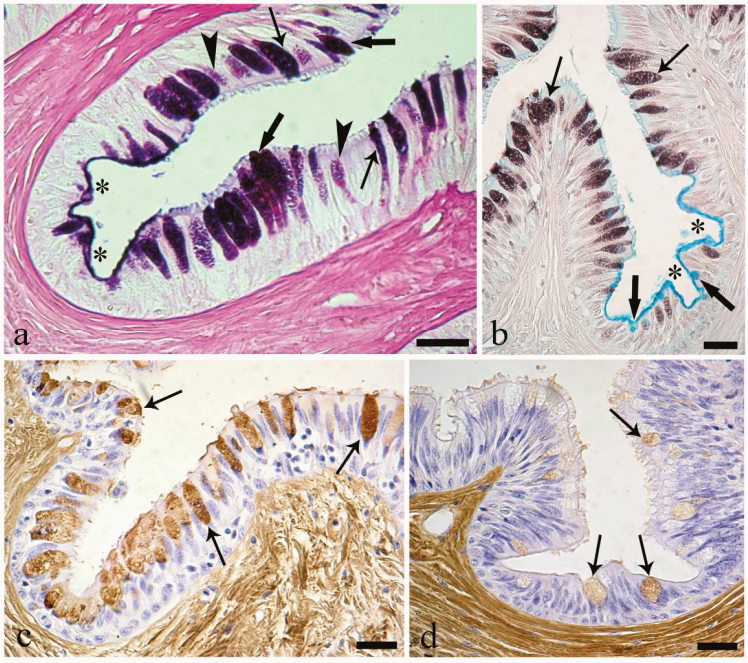

Figure 2. Mucous epithelial cells of the esophagus. (**a**) The mucous cells containing mixed mucins (acidic plus neutral) (thick arrows) are far more abundant than are acidic (thin arrows) or neutral (arrowheads) mucous cells. The epithelial brush border is unreactive, except in the crypts of the folds, rich in acidic sugar residues (asterisks). AB/PAS method. Scale bar: 20 μm. (**b**) Most of the mucous cells contain sulfated acidic mucins (thin arrows), and only a few cells are of the acidic carboxylated type (thick arrows). Note the presence of acidic carboxylated mucins in the epithelial brush border of the crypts of the folds (asterisks). HID/AB method. Scale bar: 20 μm. (**c**) The thin arrows indicate mucous cells positive to the Wheat Germ Agglutinin (WGA, N-acetyl-β-glucosamine). Scale bar: 20 μm. (**d**) The Sambucus Nigra Lectin (SNA, sialic acid-α-2,6-galactose) shows a weak positive staining of a few mucous cells (thin arrows). Scale bar: 20 μm.

Table 3. Mean number of mucous cells (±standard error) per 100,000 μm^2 of epithelial area in the alimentary canal of Galeus melastomus containing acidic carboxylated (Alcian blue-positive, AB) and acidic sulfated (high iron diamine-positive, HID) mucins. Mucous cells were counted in 5 fields (400× magnification), from 3 slides for each of the 10 Blackmouth catshark specimens (total of 150 microscopic fields).

Mucous Cell Types		Acidic Carboxylated	Acidic Sulfated	Total
Gut Regions	Esophagus	35.7 ± 1.6 (19.6%)	146.1 ± 3.7 (80.4%)	181.0 ± 4.0 (100.0%)
	Stomach	402.5 ± 6.1 (100.0%)	0.0	402.5 ± 6.1 (100.0%)
	Ascending stomach	317.9 ± 5.2 (100.0%)	0.0	317.9 ± 5.2 (100.0%)
	Proximal intestine	22.8 ± 1.3 (19.4%)	94.5 ± 3.7 (80.6%)	118.3 ± 2.9 (100.0%)
	Spiral intestine	43.0 ± 1.8 (43.0%)	57.1 ± 1.6 (57.0%)	100.6 ± 2.7 (100.0%)
	Spiral valve	34.6 ± 1.0 (36.7%)	59.7 ± 1.9 (63.3%)	94.0 ± 1.8 (100.0%)
	Distal intestine	0.0	249.8 ± 5.6 (100.0%)	249.8 ± 5.6 (100.0%)

Table 4. Mean number of mucous cells (±standard error) per 100,000 μm^2 in each region of the alimentary canal of Galeus melastomus that reacted positively to each lectin. For lectin acronyms, see Table 1. Mucous cells were counted in 5 fields (400× magnification), from 3 slides for each of the 10 Blackmouth catshark specimens (total of 150 microscopic fields).

Mucous Cell Types		ConA	DBA	PNA	SNA	UEA I	WGA
Gut Regions	Esophagus	0.0	57.7 ± 2.2	102.3 ± 3.3	2.3 ± 0.4	41.2 ± 1.6	183.2 ± 6.8
	Stomach	401.7 ± 11.3	0.0	343.2 ± 9.3	407.0 ± 11.8	0.0	0.0
	Ascending stomach	334.1 ± 8.7	0.0	402.7 ± 4.9	318.6 ± 5.8	0.0	0.0
	Proximal intestine	0.0	0.0	78.0 ± 1.9	50.1 ± 1.8	63.4 ± 2.3	55.9 ± 1.1
	Spiral intestine	0.0	0.0	42.3 ± 1.5	47.1 ± 2.2	62.3 ± 2.5	0.0
	Spiral valve	0.0	0.0	57.1 ± 1.3	49.7 ± 1.2	36.9 ± 1.4	0.0
	Distal intestine	0.0	68.5 ± 3.5	0.0	208.4 ± 6.3	62.6 ± 3.1	71.9 ± 1.6

The highest abundance of epithelial mucous cells was observed in the two regions of the stomach (Tables 2 and 3). As in the esophagus, mucous cells containing mixed mucins were more prevalent in the stomach than were mucous cells containing neutral mucins (Table 2, Figure 4a). The acidic component present in the mixed mucins was of the carboxylated type, as shown by the Alcian blue positivity of the stomach epithelium in the HID/AB reaction (Table 3, Figure 4b). The stomach glands were unstained for both histochemical sequences (AB/PAS and HID/AB) (Figure 4a,b). The cytoplasm of the stomach epithelial cells reacted weakly with the lectin Concanavalin-A (ConA) (Figures 3 and 4c). The brush border of the stomach epithelium was positive to the lectins DBA and PNA (Figure 4d,e); this lectin marked the supra-nuclear cytoplasm of the cells but not the mucous granules (Figure 4e). The glandular cells immediately below the stomach epithelium showed a reactivity for the lectin WGA (Figure 4f).

In the proximal intestine, as well as in the spiral intestine with the spiral valve, the mucous cells mainly contained mixed mucins as in the previous regions (Table 2, Figure 5a). The acidic components of the mixed mucins were prevalently sulfated (Table 3, Figure 6a,b), and the epithelial brush border showed a strong PAS reactivity (Figure 5a). In

the proximal intestine, the lectin histochemistry revealed a positive reaction of the mucous cells to PNA, SNA, UEA I, and WGA (Table 4, Figure 3). Mucous cells reactive to the lectin PNA (Figure 5b) were the most abundant (44.7%) in comparison to cells positive to the lectins SNA (24.7%), UEA I (29.7%) (Figure 5c), and WGA (31.5%) (Table 4, Figure 3). In the spiral intestine and spiral valve, approximately 23.5% of mucous cells reacted positively to the lectin SNA, 24.5% to UEA I, and 30.9% to PNA (Table 3, Figure 6c–e, respectively, Figure 3).

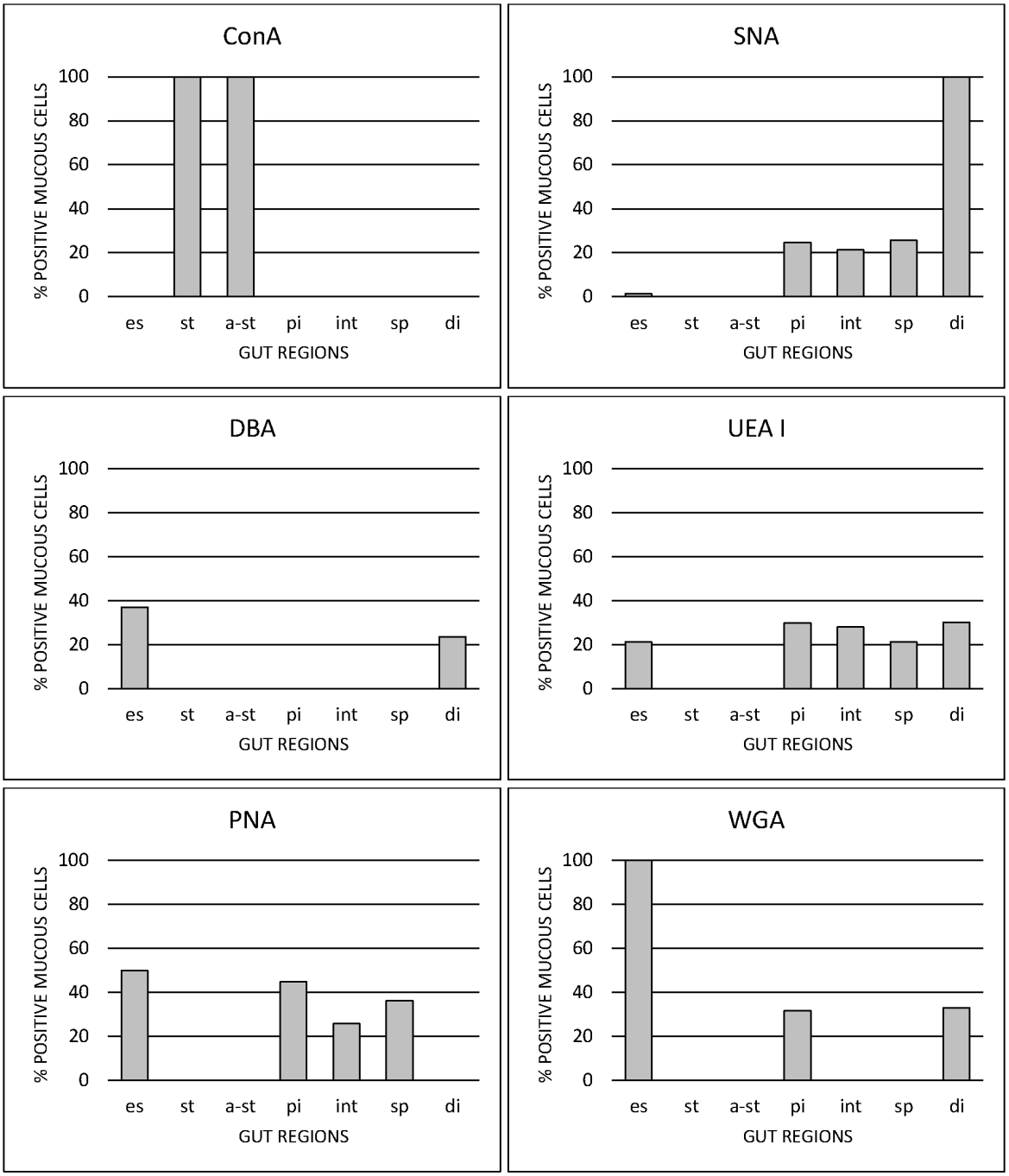

Figure 3. Histograms showing the percent of mucous cells that reacted positively to each of the six lectins in the different regions of the alimentary canal of *Galeus melastomus*. For lectin acronyms, see Table 1. Regions of the alimentary canal: es: esophagus; st: stomach; a-st: ascending part of the stomach; pi: proximal intestine; int: spiral intestine; sp: spiral valve; di: distal intestine.

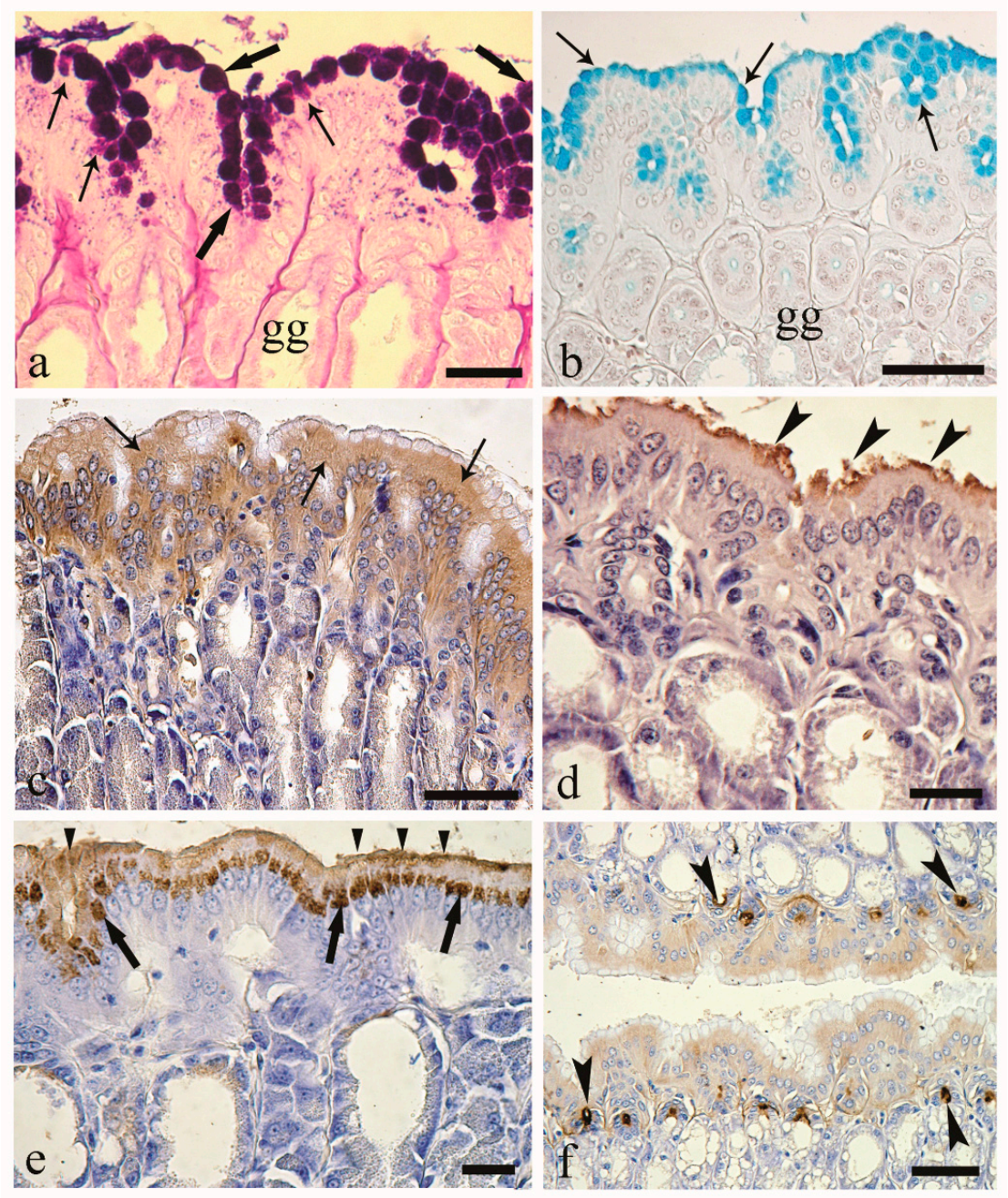

Figure 4. Mucous epithelial cells of the stomach. (**a**) Mucous cells containing mixed (thick arrows) mucins are more prevalent than mucous cells containing neutral (thin arrows) mucins. AB/PAS method. Scale bar: 20 μm. (**b**) Acidic carboxylated mucins are the only acidic mucin type present (thin arrows). HID/AB method. Scale bar: 50 μm. (**c**) The lectin Concanavalin-A (ConA, α-mannose) stained the epithelial cell cytoplasm (thin arrows) but not their apical mucous granules. Scale bar: 50 μm. (**d**) The brush border of the epithelium shows a reactivity to the lectin Dolichos Biflorus Agglutinin (DBA, N-acetyl-α-galactosamine) (arrowheads). Scale bar: 20 μm. (**e**) The supra-nuclear cytoplasm (thick arrows) of the epithelial cells is positive to the lectin Peanut Agglutinin (PNA, galactosyl(β-1,3)-N-acetyl-α-galactosamine) but their apical mucous granules are not. This lectin also stains the epithelial brush border (small arrowheads). Scale bar: 20 μm. (**f**) The stomach's cells (arrowheads) below the surface of the epithelium show a positive reaction with the lectin Wheat Germ Agglutinin (WGA, N-acetyl-β-glucosamine). Scale bar: 50 μm. In all microphotographs, the gastric glands (gg) are unstained.

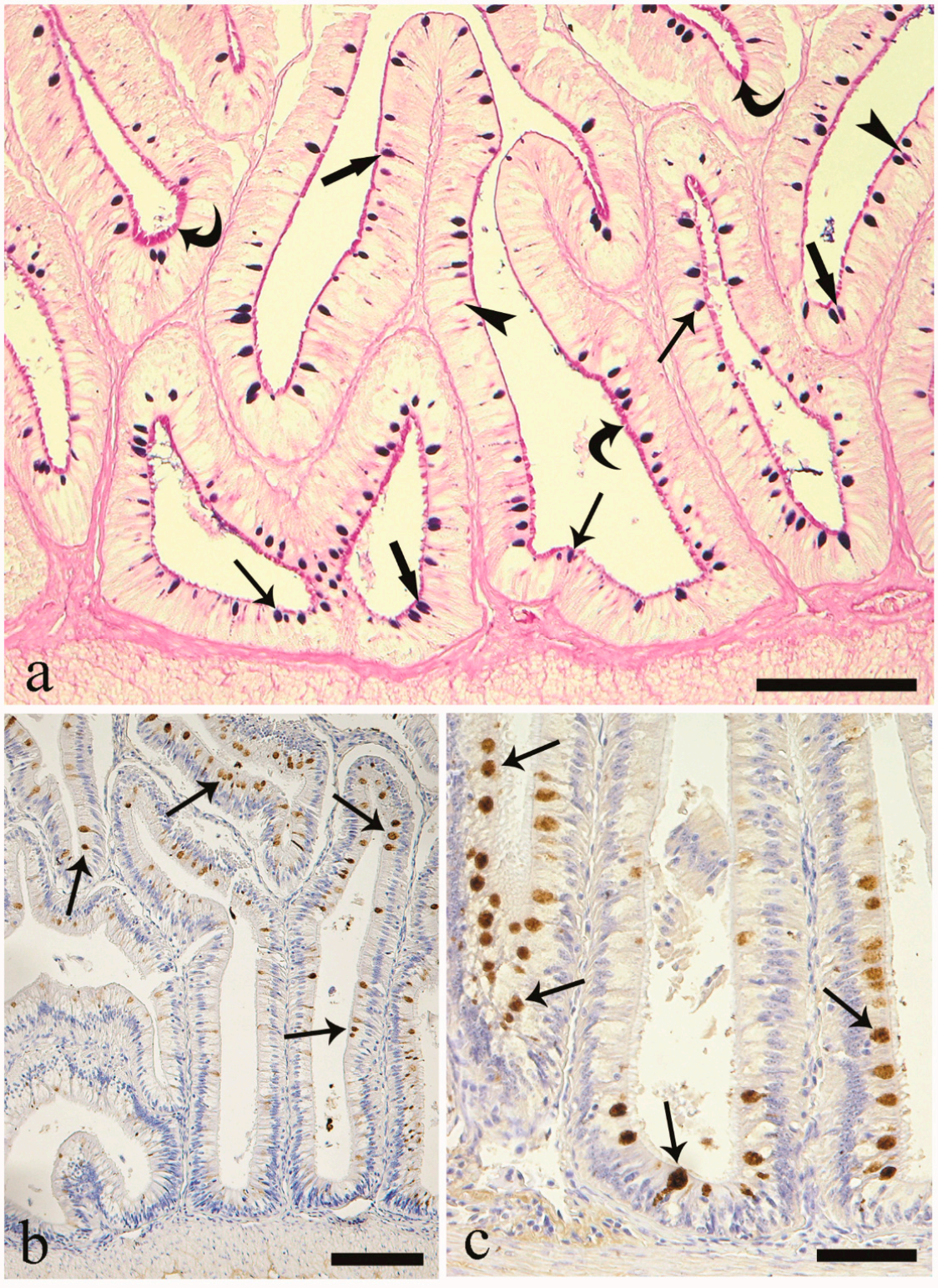

Figure 5. Mucous epithelial cells of the proximal intestine. (**a**) Mucous cells contain predominantly mixed mucins (thick arrows), and less acidic (thin arrows) and neutral (arrowheads) mucins. The epithelial brush border shows a strong reactivity to PAS (curved arrows). AB/PAS method. Scale bar: 100 µm. (**b**) The thin arrows indicate mucous cells stained with the lectin Peanut Agglutinin (PNA, galactosyl(β-1,3)-N-acetyl-α-galactosamine). Scale bar: 100 µm. (**c**) Several mucous cells (thin arrows) are positive to the lectin Ulex Europaeus Agglutinin I (UEA I, fucose-α-1,2-galactose). Scale bar: 50 µm.

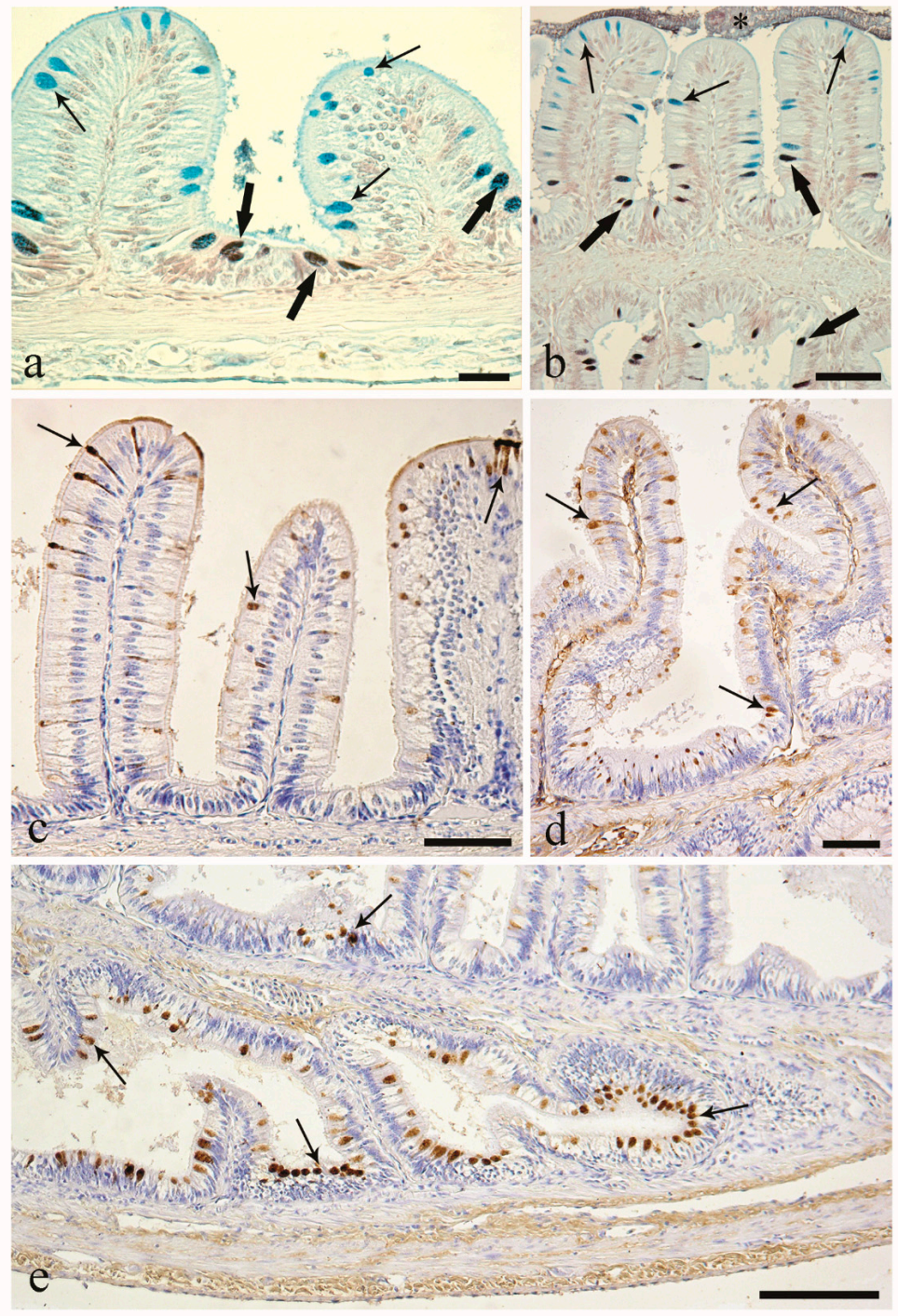

Figure 6. Mucous epithelial cells of the spiral intestine and spiral valve. (**a**) In the spiral intestine, mucous cells containing acidic carboxylated mucins (thin arrows) are slightly more prevalent than are mucous cells with acidic sulfated mucins (thick arrows). HID/AB method. Scale bar: 20 μm. (**b**) In the spiral valve, the distribution of acidic sulfated (thick arrows) and acidic carboxylated (thin arrows) mucous cells is similar as in the spiral intestine. HID/AB method. Scale bar: 50 μm. (**c**) The thin arrows show the mucous cells positive to the lectin Peanut Agglutinin (PNA, galactosyl(β-1,3)-N-acetyl-α-galactosamine) in the spiral intestine. Scale bar: 50 μm. (**d**) Mucosal folds of the spiral valve with mucous cells reactive to the Sambucus Nigra Lectin (SNA, sialic acid-α-2,6-galactose) (thin arrows). Scale bar: 50 μm. (**e**) Section of the intestine with the spiral valve with several mucous cells positive to the lectin Ulex Europaeus Agglutinin I (UEA I, fucose-α-1,2-galactose) (thin arrows). Scale bar: 100 μm.

The distal intestine showed a high number of mucous cells with a prevalence of mixed mucin-positive cells (Table 2, Figure 7a). The acidic components of mucins were sulfated, and no mucous cells containing carboxylated acidic mucins were encountered (Table 3, Figure 7b). In the distal intestine, all mucous cells were slightly positive to the lectin SNA (Table 4, Figures 3 and 7c), while mucous cells reactive to the lectins DBA, UEA I, and WGA were 23.5%, 30.1%, and 33.0% of the mucous cell population, respectively (Table 4, Figures 3 and 7d,e).

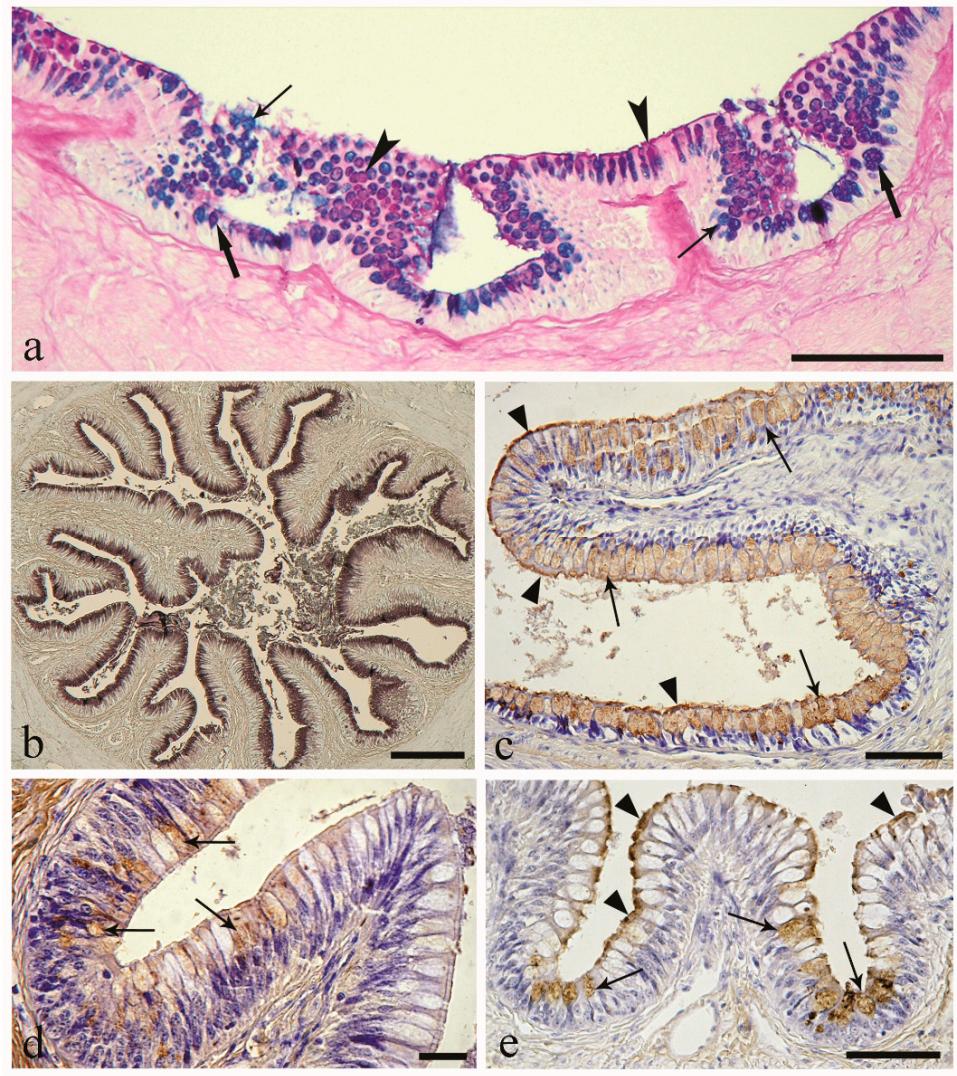

Figure 7. Mucous epithelial cells of the distal intestine. (**a**) Micrograph shows the high number of mucous cells in this region containing mixed (thick arrows), acidic (thin arrows), and neutral (arrowheads) mucins. AB/PAS method. Scale bar: 100 µm. (**b**) Transverse section of the whole rectum, showing mucous cells containing only sulfated acidic mucins. HID/AB method. Scale bar: 200 µm. (**c**) The thin arrows indicate mucous cells weakly positive to the Sambucus Nigra Lectin (SNA, sialic acid-α-2,6-galactose). The epithelial brush border (small arrowheads) shows a variable stain intensity to the lectin SNA. Scale bar: 50 µm. (**d**) Several mucous cells (thin arrows) reactive to the lectin Ulex Europaeus Agglutinin I (UEA I, fucose-α-1,2-galactose). Scale bar: 20 µm. (**e**) In the distal intestine, few mucous cells (thin arrows) and the epithelial brush border (small arrowheads) are positive to the lectin Wheat Germ Agglutinin (WGA, N-acetyl-β-glucosamine). Scale bar: 50 µm.

4. Discussion

The mucin pattern of the alimentary canal of vertebrates depends on several factors, including environmental conditions and dietary habits [10]. The pattern seen in *G. melasto-*

mus is a mixture of acidic and neutral mucins prevalent throughout the tract, with greatest abundance in the stomach. Acidic mucins predominate in the distal intestine, and mucous cells secreting neutral mucins (PAS-positive) are most abundant in the stomach. For comparison, the estuarine stingray *Himantura signifier* shows a prevalence of mucous cells with acidic mucins in the esophagus and distal intestine, and a high number of mucous cells with neutral mucins in the stomach [19]. In the White sturgeon, *Acipenser transmontanus*, and Siberian sturgeon fry, *Acipenser baerii*, the mucous cells showed mainly a mixture of acidic and neutral mucins in the esophagus, neutral mucins in the stomach, and acidic mucins in the spiral and distal intestine [10,25]. The acidic components of mucus, especially in the esophagus and distal intestine, act as a lubricant facilitating the transit of food and digesta and protecting the epithelium from mechanical damage from the passage of harsh material [26–30].

In the stomach, neutral mucins cooperate with digestive enzymes to transform food into chyme [14,26–29,31], and are involved in the absorption of small digestible molecules [25,28,31]. Moreover, neutral mucosubstances protect the gastric mucosa from the harmful effects of hydrochloric acid and digestive enzymes secreted from gastric glands [25,28,32,33]. In the stomach of *G. melastomus*, acidic mucins were exclusively of the carboxylated type, rich in sialic acid, which facilitates the mixing of the food in the lumen [28]. Indeed, sialic acids prevent the attachment of viruses, bacteria, and other pathogens to the mucosal layer [32,34,35].

In the alimentary canal of the Blackmouth catshark, acidic sulfated mucins (detected as HID-positive mucous cells) were prevalent in the esophagus, the proximal, and the spiral intestine, whilst the distal intestine possessed only acidic sulfated mucins. In the intestine of two teleost species, *Salvelinus alpinus* and *Salmo salar*, the presence of sialic acid residues associated with sulfated mucins was detected by the AB pH1.0 histochemical method, resulting in a false positive [36]. Similarly, in the regions of the alimentary canal of *G. melastomus*, the sulfated HID-positive mucins could be associated with sialic acid residues. Acidic sulfomucins are more viscous than carboxylated mucins are and highly abundant in the distal region of the fish alimentary canal [10,15,25,29,37,38]. According to Buddington and Doroshow [39], the distal region of the White sturgeon is where the most nutrients are taken up, most likely due to the high abundance of acidic sulfomucins useful for trapping small peptides or peptide fragments and able to regulate water and ion uptake [10,15,29,30,40].

The spiral intestine of elasmobranchs shows a unique twisted fold of the intestinal mucosa that significantly increases the absorptive surface [1,7,8]. Furthermore, the spiral intestine of sharks dramatically slows the passage of digesta to increase absorption [19,41–43]. Andrews and Young [44] reported a retrograde muscle peristalsis that retained food for a longer time in the intestine of the shark *Scyliorhinus canicula*. Slowing the passage of digesta presumably enables sharks to absorb the most nutrients from their infrequent meals [42,45]. Nonetheless, a large quantity of food in the spiral intestine might increase bacterial charge and consequently produce more fermentation. Thus, acidic sulfated mucins are necessary to strengthen the mucosal barrier against any pathogenic microorganisms [27,30,46].

In the alimentary canal of *G. melastomus*, the epithelial brush border was positive for neutral mucins at the surface of proximal and spiral intestinal folds, indicating that the epithelial brush border plays a role in absorption, especially in the spiral intestine [25,28,29,31]. In contrast, the brush border of the distal intestine contained both acidic and neutral mucins, making it reasonable to suggest that, in this region, the acidic mucins trap any nutrient molecules not captured in the spiral intestine and the neutral mucins help to absorb the above nutrient molecules.

In the mucous cells of the alimentary canal, different carbohydrate patterns of secreted mucins are related to the physiological activities of each region [14–17,27]. The mucous cells of the stomach presented α-mannose (lectin ConA) residues in their cytoplasm, under the mucous granules. This sugar residue was reported in the stomach epithelium of the

Stripped weakfish, *Cynoscion guatucupa* [29]. The presence of α-mannose indicates the absorption of carbohydrate by the digestive epithelium [47].

In the current study, mucins with N-acetyl-α-galactosamine (NAcGal) residues (lectin DBA) were observed in the mucous cells of the esophagus, distal intestine, and in the brush border of the stomach epithelium. NAcGal residues have also been found in the esophageal and intestinal mucous cells of *A. baerii* [25], *Esox lucius* [17], and the esophagus and stomach of several fish species [26,27,37,48]. Spicer and Schultze [49,50] suggested that NAcGal residues as components of the apical plasmalemma reveal active movement of ions and fluids across the membrane.

Mucins with galactosyl(β-1,3)-N-acetyl-α-galactosamine (Gal,β-1-3-NAcGal) residues (lectin PNA) were noticed in the mucous cells of the esophagus and the proximal and spiral intestine of the Blackmouth catshark. In the stomach, the glycoconjugates reactive to the lectin PNA were located in the supra-nuclear region of the cytoplasm, and below the mucous granules. In this region of the cell, the Golgi apparatus is well-developed and O-glycosylation of mucins takes place [50]. In another shark, *S. canicula*, Gal,β-1-3-NAcGal residues were documented in the mucous cells and brush border of the spiral valve [51]. Mucous cells reactive to the lectin PNA have been noted in the proximal intestine of *Chondrostoma nasus* [37], and in the stomach and intestine of *E. lucius* [17]. The importance of Gal,β-1-3-NAcGal residues in mucus for detection and elimination of pathogens was mentioned in Redondo and Alvarez-Pellitero [52].

Mucous cells containing sialic acid-α-2,6-galactose residues (lectin SNA) were distributed in the alimentary canal of *G. melastomus*, with a predominance in the two stomach regions and in the distal intestine. In the common carp *Cyprinus carpio*, experimental infection with *Aeromonas hydrophila* was reported to increase the prevalence of mucous cells containing sialic acid residues [13]. In the secreted mucus of germ-free rats inoculated with microbiota flora from conspecifics, an increased amount of sialic acid residues was registered after inflammation of the colonic mucosa [53]. With reference to the teleost digestive tract, the presence of rich sialic acid mucins was considered to indicate protection of the mucosa from pathogens [37].

Mucins with fucose-α-1,2-galactose (lectin UEA I) residues were encountered in the esophagus and the three intestinal regions of the Blackmouth catshark. Similar results have been seen in the mucous cells of the esophagus of *A. baerii* [25] and in the intestine of several teleost species [26,54,55]. In the intestine of mammals, the secretion of fucose-glycosylated mucins increased mucus viscosity and reduced the motility of most bacteria [56–58]. Highly fucose-glycosylated mucins are considered defensive against bacteria; microorganisms are trapped in the mucins and expelled with the feces [13].

In this study, mucous cells containing N-acetyl-β-glucosamine (NAcGlu) residues (lectin WGA) were detected in the esophagus, the proximal and distal intestine, and along the line formed by the epithelial cells of the crypts, near the more superficial stomach gland cells (see Figure 4f). Mucins with NAcGlu sugar residues were reported in the mucous cells of the pike intestine [17], in the esophageal mucous cells of the *A. baerii* [25], as well as in the spiral valve of the shark *S. canicula* [51]. The presence of NAcGlu in mucins was associated with a major acidification level of mucus [46,59]. Among the prey of sharks, some crustaceans and mollusks have exoskeletons and shells that require more time to digest compared to soft prey [4]. In the distal intestine of the shark *Sphyrna tiburo*, N-Acetyl-β-D-glucosaminidase activity was about 60 times higher than in most other fish [42]. The enzyme spike was of microbial origin [42,60] and functioned to break down the chitinous exoskeletons of crustacean prey [60]. Jhaveri et al. [42] stated that microbial digestion is common in fish that consume prey with chitinous exoskeletons; our results on Blackmouth catshark concur with that statement. The presence of WGA-positive mucins in the distal intestine of *G. melastomus* might also be due to a high level of N-acetyl-β-D-glucosaminidase produced by bacteria of the microbiome. Further studies are needed to test that possibility.

5. Conclusions

In summary, this study of the alimentary canal of the Blackmouth catshark revealed that acidic mucins were more prevalent than neutral mucins. Acidic sulfated mucins were more abundant than carboxylated mucins, especially in the intestinal regions, which favors nutrient absorption [10,15,27,29,30,39,40]. The mucins of the alimentary canal were rich in Gal,β-1-3-NAcGal, sialic acid, and α-fucose residues, which confer protection to the mucosa. In the distal intestine of *G. melastomus*, the occurrence of NAcGlu residues could be due to the spike of N-acetylglucosaminidase previously reported in this region in other catshark species that feed on chitinous prey [42,61]. This record provides for the first time data on the number of mucous cells and their distribution in each region of the alimentary canal of a shark. Our histochemical results confirm that each region, based on its function, requires a specific type of secretion. It is hoped that the evidence presented will help to develop similar information for other elasmobranch species; our findings indicate the need for further work to better understand the alimentary canal physiology of this ancient vertebrate group.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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