



CAMPUS DE SÃO JOSÉ DO RIO PRETO

**Ultraestrutura e Citoquímica
do Tubo Digestivo de *Hemisorubim platyrhynchos*
(Teleostei, Siluriformes, Pimelodidae)**

Claudemir Kuhn Faccioli

DOUTORADO



**PÓS GRADUAÇÃO
EM BIOLOGIA ANIMAL**

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Claudemir Kuhn Faccioli

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Orientador: Prof. Dr. Carlos Alberto Vicentini

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Legendas: ec-enterócitos, g-grânulos, seta-complexo de Golgi. Barra de escalas: A: 1µm, B: 500nm, C: 1µm, D: 500nm.....98

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Resumo

O conhecimento das características morfológicas do tubo digestivo, associado aos processos de digestão e absorção, é fundamental para a compreensão da fisiologia e nutrição de peixes, auxiliando no planejamento de dietas para proporcionar melhorias no manejo e sucesso em piscicultura. Assim, o objetivo do presente trabalho foi analisar as características ultraestruturais e citoquímicas do tubo digestivo de exemplares adultos de *Hemisorubim platyrhynchos* para embasar o cultivo em pisciculturas e preservação da espécie. *H. platyrhynchos* é uma espécie carnívora, distribuída nas bacias hidrográficas do Orinoco, Amazonas, Paraguai, Uruguai e Paraná. Este peixe apresenta grande potencial de consumo, devido ao tamanho dos animais e à qualidade da carne. Também é utilizado na piscicultura ornamental, devido ao padrão de coloração. Contudo, esta espécie encontra-se ameaçada de extinção no estado de São Paulo, apresentando estoque reduzido e populações isoladas. Assim, trinta exemplares foram eutanasiados e dissecados para a retirada de fragmentos do esôfago, estômago (*ventrículo gástrico*) com regiões cárdica, fúndica e pilórica e intestino (regiões anterior, medial, posterior e reto). Para os estudos ultraestruturais, fragmentos foram fixados em solução de Karnovsky modificada, pós-fixados em tetróxido de ósmio e destinados à rotina de inclusão de microscopia eletrônica de transmissão. Para os estudos citoquímicos, fragmentos de tecido foram fixados em soluções apropriadas e destinados para as seguintes técnicas citoquímicas: impregnação com tetróxido de ósmio e iodeto de zinco – ZIO (visando a análise de organelas membranosas); método ósmio-imidazol (para detecção de inclusões lipídicas), método para detecção de fosfatase ácida e alcalina e método para detecção de glicoconjugados ácidos (vermelho de rutênio). Essas técnicas possibilitaram uma análise detalhada das características celulares do epitélio de revestimento, auxiliando na compreensão da fisiologia celular ao longo do tubo digestivo de *H. platyrhynchos*.

Palavras-chave: camada mucosa, microvilosidades, morfofisiologia, bagre carnívoro.

Abstract

The knowledge of morphological characteristics of the digestive tract, associated with the processes of digestion and absorption, it is essential to understanding the physiology and nutrition of fish, assisting in planning diets to provide better management and success in farming. Thus, the aim of this study is to analyze the ultrastructural and cytochemical features of the digestive tract of adult specimen of Hemisorubim platyrhynchos to support cultivation in fish farms and preservation of the species. H. platyrhynchos is a carnivorous specie, distributed in the basins of the Orinoco, Amazonas, Paraguay, Uruguay and Paraná. This fish presents great potential for consumption, due to the animal size and meat quality. It is also used for ornamental fish due to the staining pattern of this species. However, this species is endangered in the São Paulo state, with reduced inventory and isolated populations. Thus, thirty specimens were killed and dissected to remove fragments of the esophagus, stomach (gastric ventricle) with cardiac, fundic and pyloric regions and intestine (anterior, middle, posterior and rectum). For ultrastructural studies, fragments were fixed in modified Karnovsky solution, post-fixed in osmium tetroxide and destined for inclusion in the transmission electron microscopy routine. For cytochemical studies, tissue samples were fixed on appropriate solutions and destined for the following cytochemical techniques: impregnation with zinc iodide-osmium tetroxide - ZIO (targeting analysis of membranous organelles), method osmium-imidazole (to detect of lipid droplets), method for detection of alkaline and acid phosphatase and method for detection of acid glycoconjugates (ruthenium red). These techniques enabled a detailed analysis of the cellular characteristics of the epithelial lining, aiding in the understanding of cell physiology along the digestive tract of H. platyrhynchos.

Keywords: mucosa layer, microvilli, morphophysiology, carnivorous catfish.

1. Introdução Geral

1.1. Tubo Digestivo em Peixes

Os peixes constituem o grupo mais diversificado entre os vertebrados e, em especial, a região Neotropical apresenta uma das faunas mais diversificadas de peixes de água doce do mundo (Lowe-McConnell, 1999). Porém, de acordo com Bressan et al. (2009), a deterioração do ambiente natural tem provocado aumento no número de espécies de peixes ameaçadas de extinção. Assim, o cultivo em pisciculturas é essencial para a manutenção e preservação de espécies que vem sofrendo queda acentuada em seus estoques naturais. Neste sentido, Castro et al. (2002) ressaltam que pesquisas morfofisiológicas do tubo digestivo em peixes são indispensáveis para embasar e viabilizar a criação em pisciculturas, aumentando a produtividade, diminuindo os custos de produção e, principalmente, preservando as espécies.

O tubo digestivo em peixes, segundo a literatura especializada, encontra-se dividido em cavidade bucofaríngea, esôfago, estômago e intestino, sendo geralmente constituído pelas seguintes túnicas histológicas: mucosa, submucosa, muscular e serosa (Gonçalves et al., 2013). A túnica mucosa do esôfago apresenta características muito distintas entre espécies, sendo observado desde epitélio colunar simples em *Chelmon rostratus* (Tan e Teh, 1974), pseudoestratificado em *Leporinus taeniofasciatus* (Albrecht et al., 2001), estratificado pavimentoso em *Pseudoplatystoma corruscans* (Cal, 2006), até epitélio do tipo estratificado colunar em *Pelteobagrus fulvidraco* (Cao e Wang, 2009). Ainda, Díaz et al., (2003) relataram em *Engraulis anchoita* que a região cranial do esôfago é revestida por epitélio estratificado pavimentoso, enquanto que a região caudal apresenta epitélio colunar simples.

Os principais tipos celulares evidenciados no epitélio esofágico são as células epiteliais e as células secretoras (Elbal e Agulleiro, 1986; Sarasquete et al., 2001; Carrassón et al., 2006; Naguib et al., 2011). Em relação às células secretoras, há divergência quanto à nomenclatura, sendo classificadas como caliciformes (Sarasquete et al., 2001; Cao e Wang, 2009; Hernández et al., 2009) ou mucosas (Tibbetts, 1997; Domeneghini et al., 2002; Díaz et al., 2003; Leknes, 2011). Porém, de acordo com Góes e Taboga (2005), o termo células caliciformes é aplicado a diversos tipos celulares que, mesmo apresentando algumas variações fenotípicas durante sua diferenciação, estabelecem como principal atividade metabólica a produção de secreção mucinogênica.

As mucossubstâncias, observadas por meio de análises histoquímicas das células caliciformes esofágicas, recobrem a superfície epitelial formando uma barreira protetora,

evitando lesões que possam ocorrer durante a passagem do alimento e, ainda, as secreções atuam na defesa física e imunológica contra microrganismos (Humbert et al., 1984; Leknes, 2011). As mucossubstâncias são responsáveis também pela lubrificação do epitélio (Tibbetts, 1997; Díaz et al., 2008), por auxiliar a digestão enzimática (Cao e Wang, 2009) e transformar o alimento em quimo (Murray et al., 1996).

Quanto às células epiteliais do esôfago, estudos ultraestruturais citam a presença de microvilli digitiformes na membrana plasmática apical (Elbal e Agulleiro, 1986; Morrison, 1987; Grau et al., 1992; Arellano et al., 2001; Carrassón et al., 2006). Essas microvilli protegem a superfície epitelial de traumas mecânicos provocados pela passagem de materiais ingeridos e também são responsáveis por acumular o muco secretado pelas células caliciformes, lubrificando o epitélio de revestimento (Humbert et al., 1984; Grau et al., 1992; Murray et al., 1994). Ainda, as células epiteliais esofágicas estão associadas à absorção de fluidos e íons (Elbal e Agulleiro, 1986; Carrassón et al., 2006). Segundo Yamamoto e Hirano (1978), a presença de muitas mitocôndrias e de espaços intercelulares está geralmente relacionada com transporte ativo de íons nas membranas laterais. De acordo com Carrassón et al. (2006), a existência de vesículas eletrólucidas associadas ao complexo de Golgi nas células epiteliais esofágicas de *Dentex dentex* pode ser evidência da existência de digestão pré-gástrica.

Do ponto de vista morfológico, o estômago ou ventrículo gástrico de teleósteos foi classificado por Bértin (1958) em: sifonal, cecal e retilíneo. Em geral, espécies carnívoras, como *Cichla temensis*, apresentam ventrículo gástrico grande e intestino curto, ao passo que espécies herbívoras, como *Tilapia heudeloti*, apresentam ventrículo gástrico curto e intestino longo (Nikolsky, 1963). Em *Rhamdia quelen*, Hernández et al. (2009) observaram que o ventrículo gástrico apresentou três regiões histologicamente distintas: cárdica (não glandular), fúndica (glandular e mais desenvolvida) e pilórica (menos desenvolvida). Em *Prochilodus lineatus*, Moraes et al. (1997) observaram que a região cárdica apresentou mucosa bem desenvolvida, repleta de glândulas tubulares em sua lâmina própria e verificaram que as regiões fúndica e pilórica eram aglandulares.

A mucosa gástrica é revestida por epitélio colunar simples, com mucossubstâncias apicais, geralmente neutras, identificadas por reações histoquímicas com ácido periódico de Schiff (Cal, 2006; Domeneghini et al., 2005; Hernández et al., 2009; Díaz et al., 2008). As mucossubstâncias são necessárias para a manutenção da parede gástrica, impedindo a

autodigestão pelos ácidos e enzimas secretados pelas glândulas gástricas (Ferraris et al., 1987; Domeneghini et al., 2005). De acordo com Pedini et al. (2005), além da função protetora, a presença de mucossustâncias neutras pode ser evidência de absorção e transporte de macromoléculas através de membranas. A presença de mucossustâncias no epitélio gástrico também foi evidenciada em *Oncorhynchus mykiss* por microscopia eletrônica de transmissão, sendo compostas por grânulos de tamanhos e formas diferentes, secretados para o lúmen gástrico por exocitose (Ostos-Garrido et al., 1993).

As glândulas gástricas em peixes são constituídas por um único tipo celular, as células oxintopépticas (Ostos-Garrido et al., 1996). Essas células apresentam o sistema túbulo-vesicular associado a muitas mitocôndrias, o que reflete a alta exigência de energia (Mattison e Holstein, 1980). O sistema túbulo-vesicular é composto por membranas diferenciadas a partir da membrana plasmática apical (Ostos-Garrido et al., 1996) e tem sido associado à produção de ácido clorídrico (Naguib et al., 2011).

Outra característica das células oxintopépticas, observadas em *Orthrias angorae* por Suíçmes e Ulus (2005), é a presença de um complexo de Golgi bem desenvolvido, mitocôndrias grandes e muitos grânulos esféricos de secreção. As células oxintopépticas de *Oncorhynchus mykiss* também apresentaram complexo de Golgi e retículo endoplasmático rugoso bem desenvolvidos (Ostos-Garrido et al., 1993). De acordo com Naguib et al. (2011), as células oxintopépticas de *Schilbe mystus* apresentaram numerosos grânulos de secreção, associados aos processos de produção de enzimas digestivas, principalmente pepsinogênio.

Em relação à mucosa intestinal, destacam-se dois tipos celulares no epitélio de revestimento: células absorptivas ou enterócitos e células caliciformes. As células caliciformes geralmente são descritas pela característica histoquímica de suas secreções e, segundo Grau et al. (1992), variam consideravelmente de acordo com a espécie estudada e com cada região do tubo digestivo. De acordo com Tibbets (1997), as células caliciformes do epitélio intestinal de *Arrhamphus sclerolepis krefftii* apresentaram mucossustâncias neutras e/ou ácidas, reveladas por meio de testes histoquímicos com ácido periódico de Schiff e alcian blue. As mucossustâncias ácidas protegem o epitélio intestinal contra degradação pela ação das glicosidases, aumentando a resistência do muco contra degradação bacteriana (Carrassón et al., 2006) e podem regular a transferência de proteínas ou de fragmentos, bem como de íons e fluidos (Petrinec et al., 2005). As mucossustâncias neutras podem fornecer cofatores requeridos para a quebra enzimática do alimento (Anderson, 1986) e também são responsáveis

pela digestão e emulsificação do alimento em quimo, quando combinadas com fosfatase alcalina (Clarke e Witcomb, 1980).

De modo geral, o intestino dos teleósteos pode ser dividido histofisiologicamente em: região anterior relacionada com a absorção de gorduras (Elbal e Agulleiro, 1986; Grau et al., 1992; Nachi et al., 1998; Arellano et al., 2002; Carrassón et al., 2006); região medial responsável pela absorção de macromoléculas proteicas (Stroband e Kroon, 1981); região posterior também relacionada com a absorção de macromoléculas proteicas, água e eletrólitos (Stroband et al., 1979; Morrison, 1987; Murray et al., 1996; Arellano et al., 2002; Carrassón et al., 2006) e, finalmente, região retal relacionada aos processos de defecação e absorção de proteínas (Murray et al., 1996, Hernandez-Blazquez e Silva, 1998). Em contrapartida, Gargiulo et al. (1998) descreveram a região anterior do intestino de *Tilapia* spp como responsável pela absorção de macromoléculas proteicas, enquanto a região posterior do intestino estaria associada à absorção de gorduras.

A caracterização ultraestrutural dos enterócitos também pode auxiliar na compreensão da função das diferentes regiões do intestino. Os enterócitos relacionados com absorção de gordura apresentaram grandes e numerosas vesículas delimitadas por membranas com conteúdo claro localizado na região supranuclear (Morrison, 1987; Grau et al., 1992; Murray et al., 1996; Carrassón et al., 2006). De acordo com Arellano et al. (2002), os enterócitos da região anterior do intestino de *Solea senegalensis* apresentaram quilomícrons, indicando absorção de gorduras. Por outro lado, regiões intestinais relacionadas com absorção proteica apresentaram enterócitos com elaborado sistema de invaginações de membrana, canais citoplasmáticos, vesículas de pinocitose e sistema lisossomal bem desenvolvido (Gargiulo et al., 1998). De acordo com Abaurrea-Equisoain et al. (1993), as membranas celulares estão envolvidas na absorção, por pinocitose, de macromoléculas de proteínas configurando uma via alternativa da digestão de proteínas em teleósteos, complementar ao ventrículo gástrico (Gargiulo et al., 1998).

Diante do exposto, verificou-se que a maioria dos estudos morfológicos em tubo digestivo de peixes aborda as características histológicas e histoquímicas. Na literatura especializada não foram encontrados muitos estudos em nível ultraestrutural e principalmente citoquímico do tubo digestivo, dificultando a associação das características morfológicas com a fisiologia digestiva. Assim, o presente trabalho visou à análise das características ultraestruturais e citoquímicas do tubo digestivo de *Hemisorubim platyrhynchos*, peixe

carnívoro de água doce, contribuindo para uma melhor compreensão da morfofisiologia do tubo digestivo de peixes Neotropicais.

1.2. Relevância do Estudo

O presente trabalho analisou as características ultraestruturais e citoquímicas do tubo digestivo de *Hemisorubim platyrhynchos*, com ênfase na detecção de organelas membranosas, enzimas digestivas, glicoconjugados e lipídios, permitindo o aprofundamento do conhecimento sobre a fisiologia celular do tubo digestivo em peixes de água doce.

A análise de organelas membranosas, obtidas por meio de impregnação com tetróxido de ósmio e iodeto de zinco-**ZIO** (Reincke e Walther, 1978), possibilitou a observação dos principais sítios de organização celular envolvidos em diversos processos de síntese, absorção e digestão. Esta metodologia também foi utilizada em peixes por Guimarães e Quagio-Grassiotto (2005) para descrever o desenvolvimento oocitário em *Serrasalmus spilopleura*.

A intensidade e a distribuição de enzimas digestivas variam de acordo com os hábitos alimentares e a morfologia do tubo digestivo de peixes (Gawlicka et al., 2002). A **fosfatase alcalina** é uma enzima transmembrana que tem sido descrita em diversos tecidos sendo amplamente evidenciada na borda em escova dos enterócitos (Gawlicka et al., 1995; Sastry, 1975; Smith et al., 2000; Hakim et al., 2006). Esta enzima é encontrada principalmente em membranas celulares nas quais ocorre transporte ativo e, quando combinada com mucossustâncias neutras, é responsável pela digestão e emulsificação do alimento em quimo (Clarke e Witcomb, 1980). Assim, a análise desta enzima permitiu uma melhor interpretação da função de cada região do tubo digestivo de *H. platyrhynchos*, em especial do intestino.

A enzima **fosfatase ácida**, responsável pela digestão intracelular, é encontrada principalmente em lisossomos, sendo considerada marcador lisossomal (Kužir et al., 2012). A pinocitose seguida por digestão intracelular pode ser complementar à digestão proteica no lúmen do estômago e intestino, como uma via alternativa da digestão em teleósteos (Gargiulo et al., 1998). Assim, a análise da ocorrência destas enzimas e também da presença de vesículas lipídicas intracelulares (**ósmio-imidazol**), auxiliou na compreensão dos processos de digestão e absorção de gorduras e proteínas.

A ocorrência de glicoconjugados é uma característica marcante do tubo digestivo de teleósteos (Díaz et al., 2008; Faccioli et al., 2014). Os glicoconjugados diferem muito entre as espécies e também ao longo do canal alimentar em peixes (Díaz et al., 2003). Os

glicoconjugados ácidos foram caracterizados no presente trabalho, possibilitando a sua associação com as diferentes funções do tubo digestivo, tais como lubrificação, proteção epitelial contra autodigestão, proteção contra patógenos, e também à digestão e absorção de nutrientes.

1.3. Espécie em Estudo

A espécie *H. platyrhynchos*, conhecida popularmente como jurupoca, pertence à ordem dos Siluriformes e à família Pimelodidae. É uma espécie carnívora encontrada nas bacias hidrográficas do Orinoco, Amazonas, Paraguai, Uruguai e Paraná, em geral nos rios de médio e grande porte (Bressan et al., 2009). A jurupoca não apresenta cuidado parental, realiza grandes migrações para reproduzir-se, geralmente entre os meses de novembro e fevereiro. A primeira maturação gonadal ocorre quando o peixe atinge cerca de 30 centímetros de comprimento (Pavanelli e Rego, 1989).

Esta espécie apresenta grande potencial de consumo, com representativo valor na produção pesqueira de águas interiores, devido ao tamanho dos animais e à qualidade da carne que apresenta coloração atraente, textura firme, baixo teor de gordura e ausência de ossos intramusculares – espinhos. Também é utilizada na piscicultura ornamental, devido ao padrão de coloração da espécie. Contudo, *H. platyrhynchos* está ameaçada de extinção no estado de São Paulo, devido à diminuição contínua da área, extensão e qualidade do habitat, e também em decorrência da sobrepesca e da construção de barragens hidrelétricas, que impedem a migração reprodutiva (Bressan et al., 2009).



Figura 1. Exemplar adulto de *Hemisorubim platyrhynchos*. Barra: 2,5cm.

1.4. Objetivos

O presente trabalho teve como objetivo analisar as características morfológicas e citoquímicas do tubo digestivo de exemplares adultos de *H. platyrhynchos*, com ênfase para:

- análise da anatomia macroscópica dos órgãos do tubo digestivo (esôfago, estômago ou ventrículo gástrico e intestino);
- análise das características histológicas dos diferentes segmentos;
- análise da superfície interna dos órgãos por meio de microscopia eletrônica de varredura;
- descrição dos tipos celulares presentes no epitélio de revestimento do tubo digestivo;
- detecção de organelas membranosas por meio da impregnação com tetróxido de ósmio e iodeto de zinco (ZIO);
- detecção de inclusões lipídicas (método ósmio-imidazol);
- análise das enzimas fosfatase ácida e alcalina por meio de técnicas citoquímicas;
- detecção de glicoconjugados ácidos (método vermelho de rutênio).

2. Referências Bibliográficas

- Abaurrea-Equisoain, M.A.; Nuñez, M.I.; Ostos-Garrido, M.V. Ultrastructural study of the distal part of the intestine of *Oncorhynchus mykiss*. Absorption of dietary protein. *Micron*, v.24, p.445-450, 1993.
- Albrecht, M.P.; Ferreira, M.F.N.; Caramasch, E.P. Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (Characiformes; Anostomidae). *Journal of Fish Biology*, v.58, p.419-430, 2001.
- Anderson, T.A. Histological and cytological structure of the gastrointestinal tract of the luderick, *Girella tricuspidata* (Pisces, Kyphosidae), in relation to diet. *Journal of Morphology*, v.190, p.109-119, 1986.
- Arellano, J.M.; Storch, V.; Sarasquete, C. A histological and histochemical study of the oesophagus and oesogaster of the Senegal sole, *Solea senegalensis*. *European Journal of Histochemistry*, v.45, p.279-294, 2001.
- Arellano J.M.; Storch V.; Sarasquete C. Ultrastructural study on the intestine of Senegal sole, *Solea senegalensis*. *Journal of Applied Ichthyology*, v.18, p.154-158, 2002.
- Bértn, L. Appareil digestif. In: Grassé, P.P. *Traité de zoologie*. Paris: Masson, v.13, p.1248-1302, 1958.
- Bressan, P.M.; Kierulff, M.C.M.; Sugieda, A.M. Fauna ameaçada de extinção no estado de São Paulo: Vertebrados. São Paulo: Fundação Parque Zoológico de São Paulo: Secretaria do Meio Ambiente, 646p, 2009.
- Cal, J.A. Histologia do tubo digestivo de Surubim-Pintado (*Pseudoplatystoma coruscans* – Agassiz, 1829). 87f. Dissertação (Mestrado em Ciências). Universidade de São Paulo, São Paulo, 2006.
- Cao, X.J.; Wang, W.M. Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. *Anatomia Histologia Embryologia*, v.38, p.254-261, 2009.
- Carrassón, M. Grau, A.; Dopazo, L.R.; Crespo, S. A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). *Histology and Histopathology*, v.21, p.579-593, 2006.
- Castro, E.F.; Fonseca, C.C.; Menin, E. Identificação de células endócrinas no aparelho digestório de *Prochilodus marggravii* Walbaum, 1792 (Pisces, Teleostei, Characiformes, Prochilodontidae). *Arquivos de Ciências Veterinárias e Zoologia*, v.5, n.1, p.71-78, 2002.
- Clarke, A.J.; Witcomb, D.M. A study of the histology and morphology of the digestive tract of the common eel (*Anguilla anguilla*). *Journal of Fish Biology*, v.16, p.159-170, 1980.
- Díaz, A.O.; García, A.M.; Devinenti, C.V.; Goldemberg, A.L. Morphological and histochemical characterization of the mucosa of the digestive tract in *Engraulis anchoita* (Hubbs and Marini, 1935). *Anatomia Histologia Embryologia*, v.32, p.341-346, 2003.
- Díaz, A.O.; García, A.M.; Goldemberg, A.L. Glycoconjugates in the mucosa of the digestive tract of *Cynoscion guatucupa*: A histochemical study. *Acta Histochemica*, v.110, p.76-85, 2008.
- Domeneghini, C.; Arrighi, S.; Radaelli, G.; Bosi, G.; Veggetti, A. Histochemical analysis of glycoconjugates secretion in the alimentary canal of *Anguilla anguilla* L. *Acta Histochemica*, v.106, p.477-487, 2005.

- Domeneghini, C.; Radaelli, G.; Bosi, G.; Arrighi, S.; Di Giancamillo, A.; Pazzaglia, M.; Mascarello, F. Morphological and histochemical differences in the structure of the alimentary canal in feeding and runt (feed deprived) cultured white sturgeons (*Acipenser transmontanus*). *Journal of Applied Ichthyology*, v.18, p.341–346, 2002.
- Elbal, M.T.; Agulleiro, B. A histochemical and ultrastructural study of the gut of *Sparus auratus* (Teleostei). *Journal of Submicroscopic Cytology*, v.18, p.335-347, 1986.
- Faccioli, C.K.; Chedid, R.A.; Amaral, A.C.; Vicentini, I.B.F.; Vicentini, C.A. Morphology and histochemistry of the digestive tract in carnivorous freshwater *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae). *Micron*, v.64, p.10-19, 2014.
- Ferraris, R.P.; Tan, J.D.; de la Cruz, M.C. Development of the digestive tract of milkfish, *Channos chanos*. *Aquaculture*, v.61, p.241-257, 1987.
- Gargiulo, A.M. Ceccarelli, P.; dall'Aglio, C.; Pedini, V. Histology and ultrastructure of the gut of the tilapia (*Tilapia* spp.), a hybrid teleost. *Anatomia Histologia Embryology*, v.27, p.89-94, 1998.
- Gawlicka, A.; Herold, M.A.; Barrows, F.T.; Noüe, J. de la; Hung, S.S.O. Effects of dietary lipids on growth, fatty acid composition, intestinal absorption and hepatic storage in white sturgeon (*Aciepnser transmontanus* R.) larvae. *Journal of Applied Ichthyology*, v.18, p.673–681, 2002.
- Gawlicka, A.; Teh, S.J.; Hung, S.S.O.; Hinton, D.E.; Noüe, J. de la. Histological and histochemical changes in the digestive tract of white sturgeon larvae during ontogeny. *Fish Physiology and Biochemistry*, 14:357–371, 1995.
- Góes, R.M.; Taboga, S.R. Células Caliciformes. In: Carvalho, H.F.; Collares-Buzato, C.B. A célula. Uma abordagem multidisciplinar. Manole ed, São Paulo, p.163-173, 2005.
- Gonçalves, L.U.; Rodrigues, A.P.O.; Moro, G.V.; Cargnin-Ferreira, E.; Cyrino, J.E.P. Morfologia e Fisiologia do Sistema Digestório de Peixes. In: Fracalossi D.M.; Cyrino J.E.P. Nutriaqua: nutrição e alimentação de espécies de interesse para a aquicultura brasileira. Aquabio ed, Florianópolis, 2013.
- Grau, A.; Crespo, S.; Sarasquete, M.C.; Gonzales De Canales, M.L. The digestive tract of the amberjack *Seriola dumerili* Risso: a light and scanning electron microscope study. *Journal of Fish Biology*, v.41, p.287–303, 1992.
- Guimarães, A.C.D.; Quagio-Grassiotto, I. Cytochemical characterization of the endomembranous system during the oocyte primary growth in *Serrasalmus spilopleura* (Teleostei, Characiformes, Characidae). *Tissue and Cell*, v.37, p.413–422, 2005.
- Hakim, Y.; Uni, Z.; Hulata, G.; Harpaz, S. Relationship between intestinal brush border enzymatic activity and growth rate in tilapias fed diets containing 30% or 48% protein. *Aquaculture*, v.257, p.420–428, 2006.
- Hernández, D.R.; Pérez Ganeselli, M.; Domitrovic, H.A. Morphology, histology and histochemistry of the digestive system of south american catfish (*Rhamdia quelen*). *International Journal of Morphology*, v.27, p.105-111, 2009.
- Hernandez-Blazquez, F.J.; Silva, J.R.M.C. Absorption of macromolecular proteins by the rectal epithelium of the Antarctic fish *Notothenia neglecta*. *Canadian Journal of Zoology*, v.76, p.1247–1253, 1998.

- Humbert, W.; Kirsch, R.; Meister, M.F. Scanning electron microscopic study of the oesophageal mucous layer in the eel *Anguilla anguilla* L. *Journal of Fish Biology*, v.25, p.117–122, 1984.
- Kužir, S., Gjurčević, E., Nejedli, S., Baždarić, B., Kozarić, Z. Morphological and histochemical study of intestine in wild and reared European eel (*Anguilla anguilla* L.). *Fish Physiology and Biochemistry*, v.38, p.625–633, 2012.
- Leknes, I.L. Histochemical studies on mucin-rich cells in the digestive tract of the Buenos Aires tetra (*Hyphessobrycon anisitsi*). *Acta Histochemica*, v.113, p.353–357, 2011.
- Lowe-McConnell, R.H. Estudos ecológicos de comunidades de peixes tropicais. 534p. São Paulo: EDUSP, 1999.
- Mattison, A.; Holstein, B. The ultrastructure of the gastric glands and its relation to induced secretory activity of cod, *Gadus morhua* (Day). *Acta Physiologica Scandinavica*, v.109, p.51–59, 1980.
- Moraes, M.F.P.G.; Barbola, I.F.; Guedes, E.A.C., Alimentação e relações morfológicas com o aparelho digestivo do “curimatá”, *Prochilodus lineatus* (Valenciennes) (Osteichthyes, Prochilodontidae), de uma lagoa do sul do Brasil. *Revista Brasileira de Zoologia*, v.14, p.169–180, 1997.
- Morrison, C.M. Histology of the Atlantic cod, *Gadus morhua*: an atlas. Part one. Digestive tract and associated organs. Canadian Special Publication of Fisheries and Aquatic Sciences. v.98, p.1–219, 1987.
- Murray, H.M.; Wright, G.M.; Goff, G.P. A comparative histological and histochemical study of the stomach from three species of *Pleuronectid*, the Atlantic halibut, *Hippoglossus hippoglossus*, the yellowtail flounder, *Pleuronectes ferruginea*, and the winter flounder, *Pleuronectes americanus*. *Canadian Journal of Zoology*, v.72, p.1199–1210, 1994.
- Murray, H.M.; Wright, G.M.; Goff, G.P. A comparative histological and histochemical study of the post-gastric alimentary canal from three species of *Pleuronectid*, the Atlantic halibut, the yellowtail flounder and the winter flounder. *Journal of Fish Biology*, v.48, p.187–206, 1996.
- Nachi, A.M.; Hernandez-Blazquez, F.J.; Barbieri, R.L.; Leite, R.G.; Ferri S.; Phan, M.T. Intestinal histology of a detritivorous (Iliophagous) fish *Prochilodus scrofa* Characiformes, Prochilodontidae. *Annales des Sciences Naturelles*, v.19, p.81–88, 1998.
- Naguib, S.A.A.; Ei-Shabaka, H.A.; Ashour, F. Comparative histological and ultrastructural studies on the stomach of *Schilbe mystus* and the intestinal swelling of *Labeo niloticus*. *Journal of American Science*, v.7, p.251–263, 2011.
- Nikolsky, G.V. The ecology of fishes. 352p. Academic Press. London, 1963.
- Ostos-Garrido, M.V.; Gonzalez-Oller, C.; Abaurrea-Equisoain, M.A. Effect of diet on gastric mucosa cells in the european eel (*Anguilla anguilla* L.). *Histochemical and ultrastructural study*. *Micron*, v.27, p.25–34, 1996.
- Ostos-Garrido, M.V.; Nuñez Torres, M.I.; Abaurrea-Equisoain, M.A. Histological, histochemical and ultrastructural analysis of the gastric mucosa in *Oncorhynchus mykiss*. *Aquaculture*, v.115, p.121–132, 1993.

- Pavanelli, G.C.; Rego, A.A. Novas espécies de protecefalídeos (Cestoda) de *Hemisorubim platyrhynchos* (Pisces) do Estado do Paraná. *Revista Brasileira de Biologia*, v.49, p.301–306, 1989.
- Pedini, V.; aall’Aglia, C.; Parillo, F.; Scooco, P. Glycoconjugate distribution in gastric fundic mucosa of *Umbrina cirrosa* L. revealed by lectin histochemistry. *Journal of Fish Biology*, v.66, p.222–229, 2005.
- Petrinec, Z.; Nejedli, S.; Kužir, S.; Opacak, A. Mucosubstances of the digestive tract mucosa in northern pike (*Esox lucius* L.) and european catfish (*Silurus glanis* L.). *Veterinary Archives*, v.75, p.317–327, 2005.
- Reinecke, M.; Walther, C. Aspects of turnover and biogenesis of synaptic vesicles at locust neuromuscular junctions as revealed by zinc iodide-osmium tetroxide (zio) reacting with intravesicular sh-groups. *The Journal of Cell Biology*, v.78, p.839–855, 1978.
- Sarasquete, M.C.; Gisbert, E.; Ribeiro, L.; Vieira, L.; Dinis, M.T. Glyconjugates in epidermal, branchial and digestive mucous cells and gastric glands of gilthead sea bream, *Sparus aurata*, Senegal sole, *Solea senegalensis* and Siberian sturgeon, *Acipenser baeri* development. *European Journal of Histochemistry*, v.45, p.267–278, 2001.
- Sastry, V.K. Alkaline and acid phosphatase in the digestive system of two teleost fishes. *Anatomischer Anzeiger*, v.137, p.159–65, 1975.
- Smith, B.J., Smith, S. A, Tengjaroenkul, B., Lawrence, T. A. Gross morphology and topography of the adult intestinal tract of the tilapia fish, *Oreochromis niloticus* L. *Cells. Tissues. Organs*, v.166, p.294–303, 2000.
- Stroband, H.W.J.; Kroon, A.G. The development of stomach in *Clarias lazera* and the intestinal absorption of protein macromolecules. *Cell and Tissue Research*, v.215, p.397–415, 1981.
- Stroband, H.W.J.; Meer, H.V.D.; Timmermans, L.P.M. Regional functional differentiation in the gut of the grass carp *Ctenopharyngodon idella*. *Histochemical Journal*, v.64, p.235–249, 1979.
- Suíçmez, M.; Ulus, E.A Study of the anatomy, histology and ultrastructure of the digestive tract of *Orthrias angorae* Steindachner, 1897. *Folia biologica (Kraków)*, v.53, 2005.
- Tan, C.K.; Teh, Y.F. The structure of the gut of a coral fish *Chelmon rostratus* Cuvier. *Okajimas Folia Anatomica Japonica*, v.51, p.63–79, 1974.
- Tibbetts, I.R. The distribution and function of mucous cells and their secretions in the alimentary tract of *Arrhamphus sclerolepis krefftii*. *Journal of Fish Biology*, v.50, p.809–20, 1997.
- Yamamoto, M.; Hirano. T. Morphological changes in the esophageal epithelium of the eel, *Anguilla japonica*, during adaptation to seawater. *Cell and Tissue Research*, v.192, p.25–38, 1978.

3. Descrição dos Capítulos

Capítulo 1

Título: Morphology and histochemistry of the digestive tract in carnivorous freshwater *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae).

Autores: Faccioli, C.K.; Chedid, R.A.; Amaral, A.C.; Franceschini-Vicentini, I.B.; Vicentini, C.A.

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Autores: Faccioli, C.K.; Chedid, R.A.; Mori, R.H.; Amaral, A.C.; Franceschini-Vicentini, I.B.; Vicentini, C.A.

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Capítulo 4

Título: Caracterização citoquímica de organelas membranosas, glicoconjugados ácidos e lipídios na mucosa do tubo digestivo de *Hemisorubim platyrhynchos* (Valenciennes, 1840).

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**Morphology and histochemistry of the digestive tract in carnivorous freshwater
Hemisorubim platyrhynchos (Siluriformes: Pimelodidae)**

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Abstract

The aim of this study was to characterize the morphology and histochemistry of the digestive tract of *Hemisorubim platyrhynchos*, a freshwater carnivorous catfish found in Neotropical region, using gross anatomy, light microscopy and transmission electron microscopy. This species presented a short and tubular esophagus with thick longitudinal folds. The esophageal mucosa was lined by stratified squamous epithelium containing epithelial cells, club cells and also numerous goblet cells, which secreted acidic and neutral mucins to protect and lubricate the epithelium. The stomach was a J-shaped saccular organ consisting of the cardiac, fundic and pyloric regions. The cardiac and fundic regions contained tubular gastric glands, whereas these glands were absent in the pyloric region. The gastric epithelial cells presented apical secretions that predominantly consisted of neutral mucins. The gastric musculature was, therefore, likely designed for retaining prey and the mechanical preparation of food. The intestine consisted of four regions: anterior, middle, posterior and rectal. The anterior intestine possessed thick folds to increase the surface area for absorption, the middle intestine was coiled and the posterior intestine presented thin folds and a thick musculature. The intestinal epithelium consisted mainly of enterocytes and goblet cells. Enterocytes were columnar cells with a PAS-positive brush border that contained lysosomes in the posterior intestine. Goblet cells were more numerous in the posterior intestine and secreted acidic and neutral mucins important for lubricating and protecting the epithelium. The rectum was lined by columnar epithelium with goblet cells and epithelial cells containing apical acidic and neutral mucins.

Keywords: digestive tract, ultrastructure, mucin, catfish, *Hemisorubim platyrhynchos*

3.1.1 Introduction

The digestive tract of fishes exhibits morphological and functional variations. According to Wilson and Castro (2010), morphological data of the digestive tract are important for understanding fish nutrition. In addition, morphological studies of the gut are necessary to understand pathological or physiological alterations (Carrassón *et al.*, 2006). Studies on Neotropical fishes have demonstrated anatomical differences between regions of the digestive tract. In general, these fishes have a short esophagus and a stomach that can be saccular or cecal (Menin and Mimura, 1992; Moraes *et al.*, 1997; Peretti and Andrian, 2008; Rodrigues and Menin, 2008; Hernández *et al.*, 2009). Variations have also been reported in the length of the intestine and the pattern of the intestinal loops (Moraes *et al.*, 1997; Seixas Filho *et al.*, 2000;

Seixas Filho *et al.*, 2001; Peretti and Andrian, 2008, Rodrigues and Menin, 2008; Hernández *et al.*, 2009).

Although significant differences can be observed macroscopically, the basic histological structure of the digestive tract is similar among species. The esophagus normally consists of a stratified epithelium that is composed mainly of epithelial cells and secretory cells (Menin and Mimura, 1993; Abaurrea-Equisoain and Ostos-Garrido, 1996; Arellano *et al.*, 2001; Hernandez *et al.*, 2009; Cao and Wang, 2009; Fishelson *et al.*, 2011; Xiong *et al.*, 2011; Germano *et al.*, 2013). The stomach presents a simple epithelium consisting of mucus-secreting columnar cells, whereas the intestine is composed of absorptive cells and goblet cells (Menin and Mimura, 1993; Albrecht *et al.*, 2001; Santos *et al.*, 2007; Hernandez *et al.*, 2009; Xiong *et al.*, 2011; Løkka *et al.*, 2013). In general, mucus-secreting cells are observed in the epithelial lining of the digestive tract of fish and present wide distribution and histochemical differences (Domeneghini *et al.*, 2005). The large amount of mucus produced by these cells is critical for maintaining the mucosa of the digestive tract. Although studies have shown that mucins are produced in fish (Tibbetts, 1997; Domeneghini *et al.*, 1998; Park and Kim, 2001; Pedini *et al.*, 2001; Marchetti *et al.*, 2006; Cao and Wang, 2009), few studies have described the histochemical characteristics of the epithelial lining of the digestive tract in Neotropical freshwater species (Leknes, 2010; Leknes, 2011).

Hemisorubim platyrhynchos belongs to the family Pimelodidae and the order Siluriformes. This is a migratory species without parental care that is widely distributed in the Neotropical region, with reports indicating its presence in the Orinoco, Amazon, Paraguay, Uruguay and Paraná river basins. According to Bressan *et al.* (2009), *H. platyrhynchos* is a nocturnal carnivorous fish and there has been a reduction in the population size of this species because of habitat destruction as a result of the construction of hydroelectric dams that interrupt the flow of migration required for reproduction. This species is valuable for aquaculture because of the quality and flavor of its meat and the absence of intramuscular bones. Thus, the aim of this study was to describe the anatomical, histological, ultrastructural and histochemical characteristics of the digestive tract of *H. platyrhynchos*, with the goal of increasing available knowledge regarding of the morphofunctional aspects of digestion in carnivorous Neotropical fish.

3.1.2 Material and Methods

3.1.2.1 Animals

Twenty adult specimens of *H. platyrhynchos*, with a total body length of 35.2 ± 2.3 cm, were obtained from Pirai Pisciculture (Terenos, Mato Grosso do Sul State, Brazil). The animals were anesthetized and euthanized with an overdose of benzocaine, and then dissected with a longitudinal incision along the ventral region. The total length of the digestive tract was measured, and tissue fragments were used for morphological and histochemical studies.

3.1.2.2 Gross anatomy

Five adult specimens were used for the analysis and photo documentation of the digestive tract *in situ*. The digestive tract was then removed and dissected for analysis of the internal characteristics of the esophagus, stomach and intestine. The samples were analyzed and documented using a Leica M50 stereomicroscope (Germany) and stored in 10% formalin.

3.1.2.3 Histology

Tissue fragments from the esophagus, stomach and intestines were collected from 5 specimens and immediately fixed in Bouin's solution. After fixation, the samples were washed with 70% ethanol, dehydrated in graded ethanol solutions and embedded in historesin. Histological sections (2-3 μ m) were stained with hematoxylin-eosin (HE) and 1% toluidine blue (TB), analyzed and photo-documented using an Olympus BX50 microscope (Japan).

3.1.2.4 Mucin histochemistry

Tissue fragments from the digestive tracts of 5 specimens were collected, fixed in Bouin's solution and embedded in Paraplast. Then, 5 to 7 μ m sections were prepared and processed for the characterization of mucin. To detect neutral mucins, reactions were performed using periodic acid-Schiff (PAS) reagent. To detect acidic mucins, the sections were stained with Alcian blue (AB) at pH 1.0 and 2.5. A sequential staining technique with AB (pH 2.5) and PAS was used to detect the association of acidic and neutral mucins (Cao and Wang, 2009; Suvarna *et al.*, 2012).

3.1.2.5 Transmission electron microscopy (TEM)

Samples of the digestive tract were removed from 5 specimens and fixed for 24 hours at 4°C in a solution of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH

7.4). The samples were then post-fixed for 2 hours in 1% osmium tetroxide (pH 7.4), dehydrated in a graded acetone series and embedded in Araldite resin. Resin polymerization was completed in an oven at 60°C for 48 hours. Ultrathin sections (60 and 80 nm) were mounted on copper networks and contrasted with uranyl acetate and lead citrate. The analysis and photographic documentation were performed using a Philips CM100 transmission electron microscope (Netherlands).

3.1.2.6 Ethical note

The present study was approved by the Ethical Committee for Research of the Faculty of Sciences at São Paulo State University – UNESP, Bauru, SP, Brazil, under protocol no. 1144/46/01/10.

3.1.3 Results

3.1.3.1 Gross anatomy

The digestive tract of *H. platyrhynchos* was 17.43 ± 1.27 cm total length and consisted of an oropharyngeal cavity, esophagus, prominent stomach and short intestine with few loops (Fig. 1A). The esophagus was a short tubular organ located dorsally to the liver, and it had a thick wall and pronounced internal longitudinal folds (Fig. 1B). The stomach was a J-shaped saccular organ with a thick wall and consisted of cardiac, fundic and pyloric regions (Fig. 1B). The pyloric and cardiac areas demonstrated thick longitudinal folds, whereas the fundic region had folds with different orientations. The intestine consisted of anterior, middle, posterior and rectal regions. The anterior intestine had a thin wall and thick longitudinal folds (Fig. 1C), the middle intestine was coiled, the posterior intestine was straight with thin folds (Fig. 1D) and the rectal region had no folds.

3.1.3.2 Histology and ultrastructure

The wall of the digestive tract of *H. platyrhynchos* consisted of the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Figs. 2A-2D). The lamina muscularis mucosae was only evident in the stomach (Fig. 2B), whereas in the esophagus and intestine, continuous connective tissue was observed between the lamina propria and submucosa. The tunica muscularis of the esophagus was thick and composed of two striated muscular layers, an inner longitudinal layer and an outer circular layer (Fig. 2A). In the stomach, the tunica muscularis was thick and composed of two smooth muscular layers, an inner circular layer and outer

longitudinal layer (Fig. 2B). At the transition with the intestine, the inner circular muscle layer of the stomach was more developed, forming a pyloric sphincter. In the intestine, the folds were thicker and longer in the anterior intestine, whereas the tunica muscularis became thicker closer to the posterior intestine (Figs. 2C and 2D).

The tunica mucosa of the esophagus had a stratified squamous epithelium containing three main cellular types: epithelial cells, goblet cells and club cells (Fig. 3A). The epithelial cells appeared in all layers of the epithelium, whereas the goblet cells were concentrated in the apical region opening up to esophageal lumen. The club cells appeared in the basal region of the epithelium and were fewer or absent in the posterior region of the esophagus. TEM images indicated that epithelial cells located in the surface region displayed fingerprint-like microridges in the apical plasma membrane and numerous membrane junctions between adjacent cells, mainly consisting of desmosomes and interdigitations (Fig. 3B). The epithelial cells demonstrated a central nucleus and a cytoplasm containing mitochondria and electron-lucent vesicles (Fig. 3B). These cells were also found surrounding the goblet cells and club cells. The club cells were voluminous and elongated or rounded with a central and irregular nucleus (Fig. 3D), which was also occasionally binucleate. The cytoplasm was homogeneous, and few organelles were observed around the nucleus. The plasma membrane demonstrated numerous interdigitations (Fig. 3C). The goblet cells presented a rounded and basal nucleus with a predominance of euchromatin and perinuclear cytoplasm with developed rough endoplasmic reticulum. The esophageal goblet cells contained numerous secretory granules with shape and electron density varied. The granules presented an electron-dense core while their peripheral region was electron lucent (Figs. 3E and 3F).

The stomach of *H. platyrhynchos* was lined by a simple columnar epithelium (Figs. 4A and 4B). The epithelial cells had elongated nuclei in the basal third of the cell, and the cytoplasm contained rounded mitochondria and developed rough endoplasmic reticulum (Fig. 4C). The apical cytoplasm presented numerous electron-dense rod-shaped secretory granules with homogeneous content (Figs. 4C and 4D). Interdigitations were observed on the lateral plasma membranes (Fig. 4C). The cardiac and fundic regions contained many gastric glands, which were absent in the pyloric region (Figs. 4A and 4B). The gastric glands were tubular and composed of oxynticopeptic cells, which presented a pyramidal shape and a basal and rounded nucleus, with a predominance of euchromatin but also with distinct clumps of heterochromatin (Figs. 4E and 4F). The cytoplasm contained numerous rounded or elongated mitochondria, a

developed rough endoplasmic reticulum and many spherical and electron-lucent granules (Figs. 4F and 4G). These granules were concentrated in the apical cytoplasm, which was interspersed by a tubulovesicular system. Microvilli were also present on the apical plasma membrane (Fig. 4G).

The intestine of *H. platyrhynchos* was lined by a simple columnar epithelium consisting of enterocytes and goblet cells (Figs. 5A-5D). The goblet cells were more concentrated in the posterior intestine (Fig. 5D). In the rectum, the simple epithelium consisted of columnar cells with apical mucins and scarce goblet cells (Fig. 5E). Enterocytes exhibited a basal and elongated nucleus with a predominance of euchromatin and prominent nucleoli (Figs. 5F and 5J). Numerous rounded or elongated mitochondria and a developed rough endoplasmic reticulum were also observed in the cytoplasm (Fig. 5F). The apical cytoplasm of enterocytes also displayed spherical and electron-dense lysosomes, which were increased in number compared to the posterior intestine (Fig. 5H). The plasma membrane contained numerous apical microvilli. The goblet cells had a thin base and wide apex (Figs. 5G and 5I). The basal nucleus had an irregular shape and featured a prominent nucleolus and clumps of heterochromatin (Fig. 5J). The cytoplasm presented rough endoplasmic reticulum around the nucleus and numerous secretory granules, which varied in their electron density (Figs. 5G and 5I).

3.1.3.3 Mucin histochemistry

Histochemical analysis of epithelial mucins in the digestive tract of *H. platyrhynchos* revealed two main types of secretory cells: goblet cells in the esophagus and intestine, and epithelial cells in the stomach and rectum (Table 1). Neutral mucins (PAS-positive) were present in both cell types from the beginning of the esophagus to the end of the rectum and exhibited a strong reaction (Figs. 6A, 6D, 6F and 6H). Acidic mucins (AB positive) were also detected but showed different reaction intensities (Figs. 6B and 6G). The esophageal and intestinal goblet cells exhibited an intense reaction to AB pH 2.5, revealing a large amount of carboxylated mucins, except at the end of esophagus, near the stomach. The acidic mucins formed a gel on the epithelial esophageal surface (Fig. 6B). In addition, acidic sulfated mucins (AB pH 1.0) were concentrated on the goblet cells in the initial region of the esophagus (Fig. 6B), whereas in the intestine this mucins presented a higher concentration in the posterior intestine. Gastric epithelial cells contained few acidic mucins, whereas rectal epithelial cells were rich in acidic mucins (Table 1). The sequential AB+PAS technique revealed a bluish-

purple coloration of esophageal and intestinal goblet cells (Figs. 6C and 6D), except in the regions near the stomach, where this reaction produced a predominant reddish color. Club cells and epithelial cells of the esophagus showed no reaction to PAS and AB (pH 1.0 and 2.5). In addition, a PAS-positive reaction was observed in oxynticopeptic cells of the gastric glands, in the brush border of enterocytes and in the lamina propria and submucosa of all organs analyzed.

3.1.4 Discussion

The digestive tract of *H. platyrhynchos* demonstrated many characteristics typical of carnivorous fish (Kapoor *et al.*, 1975), including a short esophagus with well-developed musculature, a large and extendable stomach and a short and slightly coiled intestine. Internally, the esophageal folds were thick and presented a longitudinal orientation, which likely enables the distention of the organ to ingest prey and facilitate the passage of food (Park and Kim, 2001; Rodrigues and Menin, 2008). The stomach of *H. platyrhynchos* was a J-shaped saccular organ with a thick wall and developed musculature. Internally, it was composed of thick longitudinal folds in the cardiac region and folds with different orientations in the fundic region. According to Rodrigues and Menin (2008), these gastric folds allow the retention of prey in the stomach, which favors more efficient mixing with digestive fluids. In addition, according to Løkka *et al.* (2013), the thickness of the gastric wall is related to the function of the region, as a strong muscular wall is necessary for the mechanical preparation of food. Macroscopic observations of the intestine of *H. platyrhynchos* revealed the presence of more developed intestinal folds in the anterior intestine. In *Pelteobagrus fulvidraco*, Cao and Wang (2009) associated the intestinal folds with the retention time of food. According to Løkka *et al.* (2013), the first segment of the intestine in teleosts is the main site of nutrient absorption, and the intestinal folds are, therefore, important for increasing the surface area for absorption.

The microscopic characteristics of the digestive tract of *H. platyrhynchos* revealed that the esophageal mucosa was lined by a stratified epithelium composed of epithelial cells, goblet cells and club cells. Numerous membrane junctions were present in the epithelial cells, mainly desmosomes and interdigitations. These membrane junctions are important for maintaining the esophageal mucosa and providing mechanical strength to the epithelium (Wilson and Castro, 2010). To facilitate the passage of food, the surface of epithelial cells of the esophageal epithelium possessed fingerprint-like microridges, which have also been described by several authors (Sperry and Wassersug, 1976; Soares *et al.*, 1995, Arellano *et al.*, 2001; El Hafez *et al.*, 2013), and are responsible for protecting the epithelial surface against mechanical injury and

anchoring the mucins secreted by goblet cells, thereby forming a lubricated epithelial surface for passage of food (Sperry and Wassersug, 1976; Humbert *et al.*, 1984; Grau *et al.*, 1992; Abaurrea-Equisoain and Ostos-Garrido, 1996).

Goblet cells were abundant in the esophageal epithelium of *H. platyrhynchos*. According to Scocco *et al.* (1998), the large amount of mucosubstances produced in the esophagus may be explained by the absence of salivary glands in fish. Thus, the mucosubstances secreted by goblet cells may protect the epithelium of the digestive tract. Ultrastructural observations revealed that esophageal goblet cells contained numerous secretory granules with shape and electron density varied. The granules presented an electron-dense core while their peripheral region was electron lucent. This distinct feature was not observed in secretory granules of other regions of the digestive tract of *H. platyrhynchos*. In addition, the histochemical reactions showed that the secretion granules were PAS-positive and AB-positive, indicating the presence of acidic and neutral mucins along the entire esophagus of *H. platyrhynchos*, with the exception of the transition region with the stomach. Several studies have linked the presence of acidic mucins to increased viscosity of secretions (Tibbetts, 1997; Díaz *et al.*, 2008a) and epithelial lubrication to the prevention of mechanical damage and epithelial protection against pathogens (Fletcher and Grant, 1969; Humbert *et al.*, 1984; Abaurrea-Equisoain and Ostos-Garrido, 1996). In *H. platyrhynchos*, there was a predominance of neutral mucins in the transition region from the esophageal epithelium to the gastric epithelium. According to Murray *et al.* (1996), neutral mucins are related to the emulsification of food into chyme and may indicate pre-gastric digestion (Cao and Wang, 2009).

In the esophageal epithelium of *H. platyrhynchos*, there were numerous club cells, especially in the anterior region of the esophagus. Club cells have also been found mainly in the skin of other fish such as *Cyprinus carpio* (Iger *et al.*, 1994), *Liobagrus mediadiposalis* (Park *et al.*, 2003) and *Arius tenuispinis* (Al-Banaw *et al.*, 2010). According to Nakamura *et al.* (2001), these cells are abundant in the skin, s, oral cavity and pharynx of *Conger myriaster* and appear in smaller numbers in the esophagus. Histochemical analyses of the club cells in the esophagus of *H. platyrhynchos* showed negative reactivity for mucins. Some studies have suggested that these cells exhibit only protein secretion (Nakamura *et al.*, 2001; Al Banaw *et al.*, 2010). Moreover, the club cells of *H. platyrhynchos* were large and demonstrated no openings to the esophageal lumen. According to Nakamura *et al.* (2001), the club cells of *Conger myriaster* undergo holocrine secretion via rupture of the cell during epithelial injury,

and these cells also form the first line of defense to protect the tissues underlying the epithelium (Chivers *et al.*, 2007). According to Godinho *et al.* (1970), club cells also provide protection to the esophageal epithelium of *Pimelodus maculatus*. In addition, Iger *et al.* (1994) reported that the club cells in the skin of *Cyprinus carpio* influence epithelial cell kinetics and are involved in removing leukocytes. Thus, the club cells present in the esophagus of *H. platyrrhynchus* may protect against epithelial injury caused when prey is ingested, which explains their higher concentration in the anterior region of the esophagus.

The gastric mucosa of *H. platyrrhynchus* was lined with a simple columnar epithelium consisting of epithelial cells rich in apical neutral mucins, similarly to other species (Ferraris *et al.*, 1987; Pedini *et al.*, 2001; Carrassón *et al.*, 2006; Cao and Wang, 2009). According to Noaillac-Depeyre and Gas (1978), mucins of the gastric epithelium are secreted by exocytosis, and according to Ferraris *et al.* (1987), they may protect the epithelium of the stomach from autodigestion processes caused by secretions produced by the gastric glands. In the present study, histochemical tests with AB also revealed the presence of acidic mucins in gastric epithelial cells, although these reactions were less intense. According to Spicer and Schulte (1992), acidic mucins may be able to form a complex with pepsin, thereby stabilizing or buffering the enzyme because of their anti-peptic activity.

In the gastric mucosa of the cardiac and fundic regions of *H. platyrrhynchus*, we observed a region with abundant gastric glands, similar to that observed in the catfish *Pelteobagrus fulvidraco* (Cao and Wang, 2009). The abundance of gastric glands observed in *H. platyrrhynchus* is likely important for digestion of prey, which is normally swallowed whole. The gastric glands were composed of a single cellular type, oxynticopeptic cells, which has also been reported for other species such as *Oncorhynchus mykiss* (Ostos-Garrido *et al.*, 1993), *Oreochromis niloticus* (Gargiulo *et al.*, 1997), *Dicentrarchus labrax* (Garcia-Hernandez *et al.*, 2001) and *Micropogonias furnieri* (Díaz *et al.*, 2008b). The oxynticopeptic cells of *H. platyrrhynchus* displayed a tubulovesicular system and numerous rounded or elongated mitochondria, a characteristic that Mattison and Holstein (1980) associated with the high energy demand of ionic exchange. According to Naguib *et al.* (2011), the production of hydrochloric acid is associated with a tubulovesicular system, which is composed of differentiated membranes derived from the apical plasma membrane (Ostos-Garrido *et al.*, 1993). The oxynticopeptic cells of *H. platyrrhynchus* also exhibited a developed rough endoplasmic reticulum and many rounded and electron-lucent granules. These characteristics have also been

reported by other studies, along with the production and secretion of pepsinogen (Ostos-Garrido *et al.*, 1993; Naguib *et al.*, 2011).

H. platyrhynchos displays a short intestine with few loops, similar to other carnivorous species (Seixas Filho *et al.*, 2001; Rodrigues and Menin, 2008). Microscopic observations showed mainly enterocytes and goblet cells in the intestinal epithelium of the species studied. The enterocytes presented a basal nucleus, numerous mitochondria and electron-dense lysosomes in apical cytoplasm. In the posterior intestine, the enterocytes possessed a higher number of lysosomes, and this region has been associated with absorption of protein macromolecules (Hernandez-Blazquez *et al.*, 2006; Borges *et al.*, 2010; Wilson and Castro, 2010; Naguib *et al.*, 2011). According to Gargiulo *et al.* (1998), the posterior region of the intestine performs pinocytosis of protein macromolecules and intracellular digestion, and the lysosomes responsible for this function also aid in gastric digestion. The enterocytes of *H. platyrhynchos* also displayed a PAS-positive brush border. According to Murray *et al.* (1996), the glycocalyx present on the brush border of enterocytes contains neutral glycoconjugates that, together with alkaline phosphatase, are involved in emulsification of food into chyme and nutrient absorption (Stroband *et al.*, 1979; Clarke and Witcomb, 1980).

In the present study, goblet cells were more abundant in the posterior intestine. The secretions of goblet cells are thought to provide protection for the epithelium and lubrication to facilitate the transit of food, as reported by Carrassón *et al.* (2006). The goblet cells of *H. platyrhynchos* exhibited PAS-positive granules, which indicated the presence of neutral mucins that may provide co-factors required for the enzymatic breakdown of food (Anderson, 1986), and AB-positive granules, which indicated the presence of acidic mucins that protect the intestinal epithelium against degradation by glycosidases (Carrassón *et al.*, 2006). The posterior intestine also demonstrated a higher concentration of sulfated mucins (AB pH 1.0) aiding in the absorption of proteins or protein fragments, ions and fluids (Petrinec *et al.*, 2005). Furthermore, the addition of sulfate radicals to glycoproteins is known to be important for increasing the resistance of mucus to bacterial degradation (Rhodes *et al.*, 1985).

The epithelial lining of the rectal region of *H. platyrhynchos* was a simple columnar, consisting of epithelial cells and few goblet cells. The epithelial cells exhibited apical secretion throughout the epithelium, a characteristic that has not been reported in the literature for other teleost species. The histochemical analysis showed that these secretions were composed of both neutral and acidic mucins. The rectal region has been associated with the final uptake of water,

ions and proteins (Murray *et al.*, 1996, Hernandez-Blazquez and Silva, 1998; Carrasón *et al.*, 2006; Elbal and Agulleiro, 1986). Therefore, the mucin-containing epithelial cells in the rectal region of *H. platyrhynchos* may be involved in fecal transit, epithelial protection and also the final absorption of substances.

Considering that *H. platyrhynchos* is a carnivorous catfish from the Neotropical region, the digestive tract likely possesses many anatomical features to facilitate the intake and retention of whole prey for subsequent digestion and nutrient absorption. Moreover, the production of acidic and neutral mucins by epithelial cells and goblet cells throughout the digestive tract of *H. platyrhynchos* may provide protection and lubrication for the epithelial surface and facilitate the passage of food. The presence of mucin in different regions of the intestine of teleosts has also been associated with the absorption and transport of macromolecules, as reported in numerous previous studies (Elbal and Agulleiro, 1986; Murray *et al.*, 1996; Scocco *et al.*, 1998; Domeneghini *et al.*, 1998; Arellano *et al.*, 1999; Petrinc *et al.*, 2005; Carrasón *et al.*, 2006).

3.1.5 Acknowledgements

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3.1.6 References

- Abaurrea-Equisoain, M.A., Ostos-Garrido, M.V., 1996. Cell Types in the Esophageal Epithelium of *Anguilla anguilla* (Pisces, Teleostei). Cytochemical and Ultrastructural Characteristics. *Micron*, 27, 419-429.
- Al-Banaw, A., Kenngott, R., Al-Hassan, J.M., Mehana, N., Sinowatz, F., 2010. Histochemical analysis of glycoconjugates in the skin of a catfish (*Arius Tenuispinis*, Day). *Anat. Histol. Embryol.*, 39, 42-50.
- Albrecht, M.P., Ferreira, M.F.N., Caramaschi, E.P., 2001. Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (Caraciformes; Anostomidae). *J. Fish Biol.*, 58, 419-430.
- Anderson, T.A., 1986. Histological and cytological structure of the gastrointestinal tract of the luderick, *Girella tricuspidata* (Pisces, Kyphosidae), in relation to diet. *J. Morphol.*, 190, 109-119.

- Arellano, J., Dinis, M.T., Sarasquete, C., 1999. Histomorphological and histochemical characteristics of the intestine of the Senegal sole, *Solea senegalensis*. Eur. J. Histochem., 43, 121-133.
- Arellano, J.M., Storch, V., Sarasquete, C., 2001. A histological and histochemical study of the oesophagus and oesogaster of the Senegal sole, *Solea senegalensis*. Eur. J. Histochem., 45, 279-294.
- Bressan, P.M., Kierulff, M.C.M., Sugieda, A.M., 2009. Fauna ameaçada de extinção no estado de São Paulo: Vertebrados. Fundação Parque Zoológico de São Paulo: Secretaria do Meio Ambiente, São Paulo, 646p.
- Borges, J.C.S., Pressionotti, L.N., Vicente Gomes, V., Cunha Da Silva, J.R.M., 2010. Lipidic and proteic absorption in digestive tract of tropical fat snook (*Centropomus parallelus*, POEY 1860). J. Exp. Mar. Biol. Ecol., 386, 39-44.
- Cao, X.J., Wang, W.M., 2009. Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. Anat. Histol. Embryol., 38, 254-261.
- Carrassón, M., Grau, A., Dopazo, L.R., Crespo, S., 2006. A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). Histol. Histopathol., 21, 579-593.
- Chivers, D.P., *et al.*, 2007. Epidermal 'alarm substance' cells of fishes maintained by non-alarm functions: possible defence against pathogens, parasites and UVB radiation. Proc. R. Soc. B, 274, 2611-2619.
- Clarke, A.J., Witcomb, D.M., 1980. A study of the histology and morphology of the digestive tract of the common eel (*Anguilla anguilla*). J. Fish Biol., 16, 159-170.
- Díaz, A.O., García, A.M., Goldemberg, A.L., 2008a. Glycoconjugates in the mucosa of the digestive tract of *Cynoscion guatucupa*: A histochemical study. Acta Histochem., 110, 76-85.
- Díaz, A.O., García, A.M., Figueroa, D.E., Goldemberg, A.L., 2008b. The mucosa of the digestive tract in *Micropogonias furnieri*: A light and electron microscope approach. Anat. Histol. Embryol. 37, 251-256.
- Domeneghini, C., Ponnelli Straini, R., Veggetti A., 1998. Gut glycoconjugates in *Sparus aurata* L. (Pisces, Teleostei). A comparative histochemical study in larval and adult ages. Histol. Histopathol., 13, 359-372.
- Domeneghini, C., Arrighi, S., Radaelli, G., Bosi, G., Veggetti, A., 2005. Histochemical analysis of glycoconjugates secretion in the alimentary canal of *Anguilla anguilla* L. Acta Histochem., 106, 477-487.
- Elbal, M.T., Agulleiro, B., 1986. A histochemical and ultrastructural study of the gut of *Sparus auratus* (Teleostei). J. Submicrosc. Cytol., 18, 335-347.
- El Hafez, E.A.A., Mokhtar, D.M., Abou-Elhamd, A.S., Hassan, A.H.S., 2013. Comparative Histomorphological Studies on Oesophagus of Catfish and Grass Carp. J. Histol., 1-10.
- Ferraris, R.P., Tan, J.D., de la Cruz, M.C., 1987. Development of the digestive tract of milkfish, *Channos chanos*. Aquaculture, 61, 241-257.
- Fletcher, T.C., Grant, P.T., 1969. Immunoglobulins in the serum and mucus of the plaice (*Pleuronectes platessa*). Biochem. J., 115, 65.

- Fishelson, L., Golani, D., Russell, B., Galil, B., Goren, M., 2011. Comparative morphology and cytology of the alimentary tract in lizard fishes (Teleostei, Aulopiformes, Synodontidae). *Acta Zool.*, 93, 308-318.
- García-Hernández, M.P., Lozano, M.T., Elbal, M.T., Agulleiro, B., 2001. Development of the digestive tract of sea bass (*Dicentrarchus labrax* L.). Light and electron microscopic studies. *Anat. Histol. Embryol.*, 204, 39-57.
- Gargiulo, A.M., Ceccarelli, P., Dall'aglio, C., Pedini, V., 1997. Ultrastructural study on the stomach of *Tilapia* spp (Teleostei). *Anat. Histol. Embryol.*, 26, 331-336.
- Gargiulo, A.M., Ceccarelli, P., Dall'aglio, C., Pedini, V., 1998. Histology and ultrastructure of the gut of the tilapia (*Tilapia* spp.), a hybrid teleost. *Anat. Histol. Embryol.*, 27, 89-94.
- Germano, R.M., Stabille, S.R., Mari, R.B., Pereira, J.N.B., Faglioni, J.R.S., Miranda-Neto, M.H., 2013. Morphological characteristics of the *Pterodoras granulosus* digestive tube (Valenciennes, 1821) (Osteichthyes, Doradidae). *Acta Zool.*, 0, 1-10.
- Godinho, H., Tokumaru, M., Ferri, A.G., 1970. Histologia do trato digestivo de *Pimelodus maculatus*, Lacépède. *Rev. Bras. Biol.*, 30, 583-593.
- Grau, A., Crespo, S., Sarasquete, M.C., Gonzales de Canales, M.L., 1992. The digestive tract of the amberjack *Seriola dumerili* Risso: a light and scanning electron microscope study. *J. Fish Biol.*, 41, 287-303.
- Hernandez-Blazquez, F.J., Silva, J.R.M.C., 1998. Absorption of macromolecular proteins by the rectal epithelium of the Antarctic fish *Notothenia neglecta*. *Can. J. Zool.*, 76, 1247-1253.
- Hernandez-Blazquez, F.J., Guerra, R.R., Kfoury Jr, J.R., Bombonato, P.P., Cogliati, B., Silva, J.R.M.C., 2006. Fat absorptive processes in the intestine of the Antarctic fish (Richardson, 1844). *Polar Biol.*, 29, 831-836.
- Hernández, D.R., Pérez Ganeselli, M., Domitrovic, H.A., 2009. Morphology, histology and histochemistry of the digestive system of south american catfish (*Rhamdia quelen*). *Int. J. Morphol.*, 27, 105-111.
- Humbert, W., Kirsch, R., Meister, M.F., 1984. Scanning electron microscopic study of the oesophageal mucous layer in the eel *Anguilla anguilla* L. *J. Fish Biol.*, 25, 117-122.
- Iger, Y., Abraham, M., Wendelaar Bonga, S.E., 1994. Response of club cells in the skin of the carp *Cyprinus carpio* to exogenous stressors. *Cell Tissue Res.*, 277, 485-491.
- Kapoor, B.G., Smit, H., Verighina, I.A., 1975. The alimentary canal and digestion in Teleosts. In: Russell F.S., Young M. (Eds.). *Advances in marine biology*. Academic Press. London, pp.109-239.
- Leknes, I.L., 2010. Histochemical study on the intestine goblet cells in cichlid and poeciliid species (Teleostei). *Tissue Cell*, 42, 61-64.
- Leknes, I.L., 2011. Histochemical studies on mucin-rich cells in the digestive tract of the Buenos Aires tetra (*Hyphessobrycon anisitsi*). *Acta Histochem.*, 113, 353-357.
- Løkka, G., Austbø, L., Falk, K., Bjerkå, I., Koppang, E.O., 2013. Intestinal morphology of the wild atlantic salmon (*Salmo salar*). *J. Morphol.*, 274, 859-76.
- 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*. *J. Fish Biol.*, 68, 1808-1821.

- Mattison, A., Holstein, B., 1980. The ultrastructure of the gastric glands and its relation to induced secretory activity of cod, *Gadus morhua* (Day). *Acta Physiol. Scand.*, 109, 51-59.
- Menin, E., Mimura, O.M., 1992. Anatomia funcional comparativa do estômago de três peixes Teleostei de hábito alimentar onívoro. *Rev. Ceres*, 39, 233-260.
- Menin, E., Mimura, O.M., 1993. Anatomia comparativa do estômago de três peixes Teleostei de água doce de hábito alimentar ictiófago. *Rev. Ceres*, 40, 203-222.
- Moraes, M.F.P.G., Barbola, I.F., Guedes, E.A.C., 1997. Alimentação e relações morfológicas com o aparelho digestivo do “curimatá”, *Prochilodus lineatus* (Valenciennes) (Osteichthyes, Prochilodontidae), de uma lagoa do sul do Brasil. *Rev. Bras. Zool.*, 14, 169-180.
- Murray, H.M., Wright, G.M., Goff, G.P., 1996. A comparative histological and histochemical study of the post-gastric alimentary canal from three species of Pleuronectid, the Atlantic halibut, the yellowtail flounder and the winter flounder. *J. Fish Biol.*, 48, 187-206.
- Naguib, S.A.A., Ei-Shabaka, H.A., Ashour, F., 2011. Comparative histological and ultrastructural studies on the stomach of *Schilbe mystus* and the intestinal swelling of *Labeo niloticus*. *J. Am. Sci.*, 7, 251-263.
- Nakamura, O., Watanabe, T., Kamiya, H., Muramoto, K., 2001. Galectin containing cells in the skin and mucosal tissues in Japanese conger eel, *Conger myriaster*: an immunohistochemical study. *Dev. Comp. Immunol.*, 25, 431-437.
- Noaillac-Depeyre, J., Gas, N., 1978. Ultrastructural and cytochemical study of the gastric epithelium in a fresh water teleostean fish *Perea fluviatilis*. *Tissue Cell*, 10, 23-37.
- Ostos-Garrido, M.V., Gonzalez-Oller, C., Abaurrea Equisoain, M.A., 1993. Histological, histochemical and ultrastructural analysis of the gastric mucosa in *Oncorhynchus mykiss*. *Aquaculture*, 115, 121-132.
- Park, J.Y., Kim, I.S., 2001. Histology and mucin histochemistry of the gastrointestinal tract of the mud loach, in relation to respiration. *J. Fish Biol.*, 58, 861-872.
- Park, J.Y., Kim, I.S., Kim, S.Y., 2003. Structure and histochemistry of the skin of a torrent catfish, *Liobagrus mediadiposalis*. *Environ. Biol. Fish.*, 66, 3-8.
- Pedini, V., Scocco, P., Radaelli, G., Fagioli, O., Ceccarelli, P., 2001. Carbohydrate histochemistry of the alimentary canal of the shi drum, *Umbrina cirrosa* L. *Anat. Histol. Embryol.*, 30, 345-349.
- Peretti, D., Andrian, I.F., 2008. Feeding and morphological analysis of the digestive tract of four species of fish (*Astyanax altiparanae*, *Parauchenipterus galeatus*, *Serrasalmus marginatus* and *Hoplias aff. malabaricus*) from the upper Paraná River floodplain, Brazil. *Braz. J. Biol.*, 68, 671-679.
- Petrinec, Z., Nejedli, S., Kužir, S., Opacak, A., 2005. Mucosubstances of the digestive tract mucosa in northern pike (*Esox lucius* L.) and european catfish (*Silurus glanis* L.). *Vet. Arhiv.*, 75, 317-327.
- Rhodes, J.M., Black, R.R., Gallimore, R., Savage, A., 1985. Histochemical demonstration of desialitation and desulphation of normal and inflammatory bowel disease rectal mucus by faecal extracts. *Gut*, 26, 1312-1318.
- Rodrigues, S.S., Menin, E., 2008. Anatomia do tubo digestivo de *Salminus brasiliensis* (Cuvier, 1817) (Pisces, Characidae, Salmininae). *Biotemas*, 21, 65-75.

- Santos, C.M., Duarte, S., Souza, T.L.G., Ribeiro, T.P., Sales, A., Araújo, F.G., 2007. Histologia e caracterização histoquímica do tubo gastrintestinal de *Pimelodus maculatus* (Pimelodidae, Siluriformes) no reservatório de Funil, Rio de Janeiro, Brasil. *Iheringia. Sér. Zool.*, 97, 411-417.
- Scocco, P., Accili, D., Menghi, G., Ceccarelli, P., 1998. Unusual glycoconjugates in the esophagus of a tilapine polyhybrid. *J. Fish Biol.*, 53, 39-48.
- Seixas Filho, J.T., Brás, J.M., Gomide, A.T.M., Oliveira, M.G.A., Donzele, J.L., Menin, E., 2000. Anatomia funcional e morfometria dos intestinos e dos cecos pilóricos do Teleostei (pisces) de água doce *Brycon orbignyanus* (Valenciennes, 1849). *Rev. Bras. Zootec.*, 29, 313-324.
- Seixas Filho, J.T., Brás, J.M., Gomide, A.T.M., Oliveira, M.G.A., Donzele, J.L., Menin, E., 2001. Anatomia funcional e morfométrica do intestino no Teleostei (Pisces), de água doce surubim (*Pseudoplatystoma corruscans* - Agassiz, 1829). *Rev. Bras. Zootec.*, 30, 1670-1680.
- Soares, T.R.S., Watanabe, I.S., Lopes, R.A., Semprini, M., Bahls, A.S., Brentegani, L.G., Satake, T., 1995. Light and scanning electron microscopic study of the digestive tract in the catfish *Pseudoplatystoma corruscans* Agassiz, 1829 (Pisces, Pimelodidae). *Braz. J. Morphol. Sci.*, 12, 101-108.
- Sperry, D.G., Wassersug, R.J., 1976. A proposed function for microridges on epithelial cells. *Anat. Rec.*, 185, 253-257.
- Spicer, S.S., Schulte, B.A., 1992. Diversity of cell glycoconjugates shown histochemically: a perspective. *J. Histochem. Cytochem.*, 40, 1-38.
- Stroband, H.W.J., Meer, H.V.D., Timmermans, L.P.M., 1979. Regional functional differentiation in the gut of the grass carp *Ctenopharyngodon idella*. *Histochem. J.*, 64, 235-249.
- Suvarna, S.K., Layton, C., Bancroft, J.D., 2012. Bancroft's Theory and Practice of Histological Techniques. Seventh ed., Churchill Livingstone, London.
- Tibbetts, I.R., 1997. The distribution and function of mucous cells and their secretions in the alimentary tract of *Arrhamphus sclerolepis krefftii*. *J. Fish Biol.*, 50, 809-820.
- Xiong, D., Zhang, L., Yu, H., Xie, C., Kong, Y., Zeng, Y., Huo, B., Liu, Z., 2011. A study of morphology and histology of the alimentary tract of *Glyptosternum maculatum* (Sisoridae, Siluriformes). *Acta Zool.*, 92, 161-169.
- Wilson, J.M., Castro, L.F.C., 2010. Morphological diversity of the gastrointestinal tract in fishes. In: Grosell M., Farrell A.P., Brauner C.J. (Eds.). *Fish physiology: the multifunctional gut of fish*. Academic Press, San Diego, pp. 1-55.

3.1.7 Figure legends

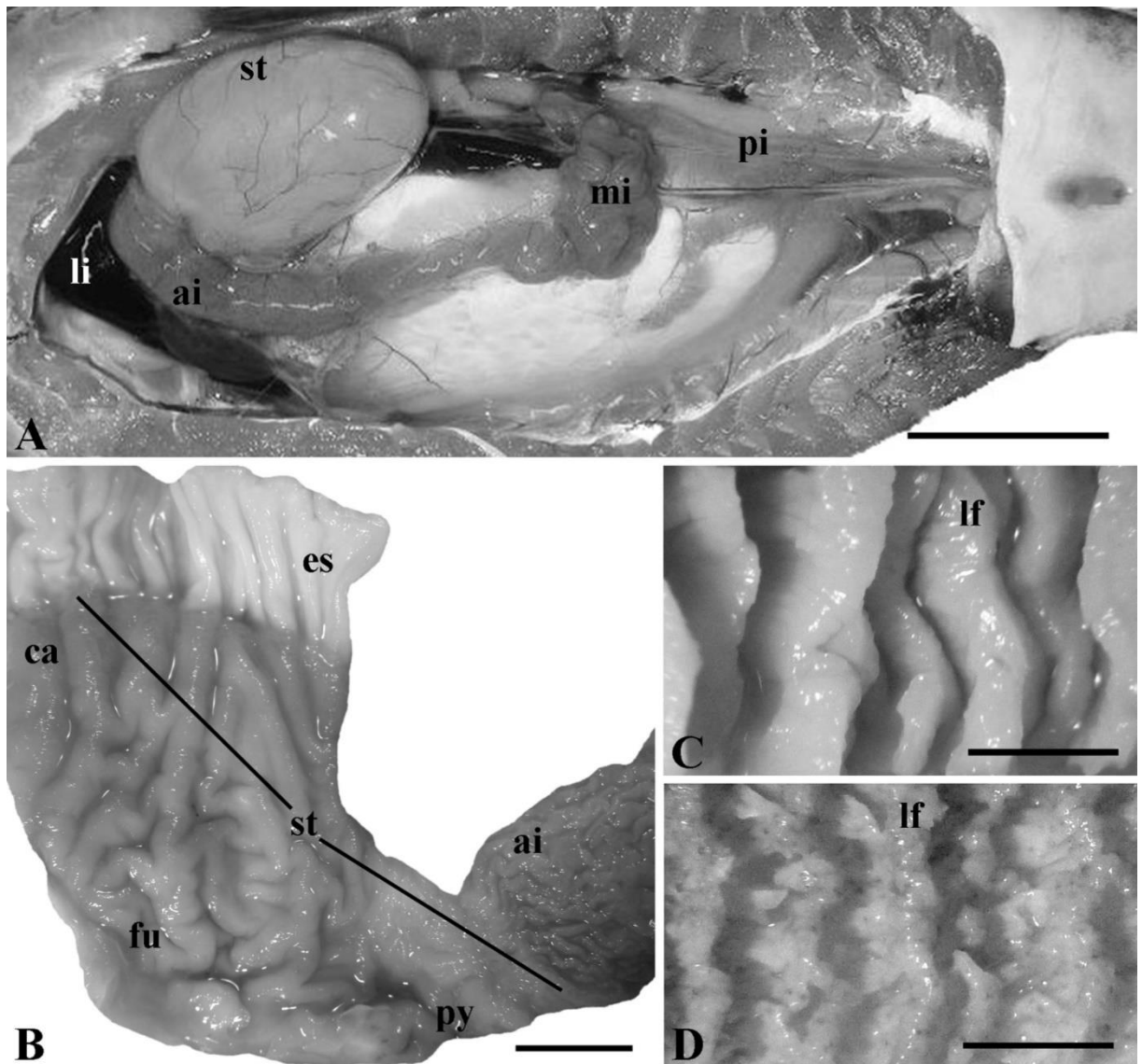


Figure 1. (A) Ventral view of peritoneal cavity of *Hemisorubim platyrhynchos* showing the organs of the digestive tract: liver (li), stomach (st), anterior intestine (ai), middle intestine (mi) and posterior intestine (pi). (B) Macroscopic image of the luminal surface of the digestive tract: esophagus (es), cardiac (ca), fundic (fu) and pyloric (py) regions of stomach (st) and anterior intestine (ai). (C) Anterior intestine, with thick longitudinal folds (lf). (D) Posterior intestine, showing the thin longitudinal folds (lf). Scale bars: A= 30mm, B= 8mm, C= 3mm, D= 1mm.

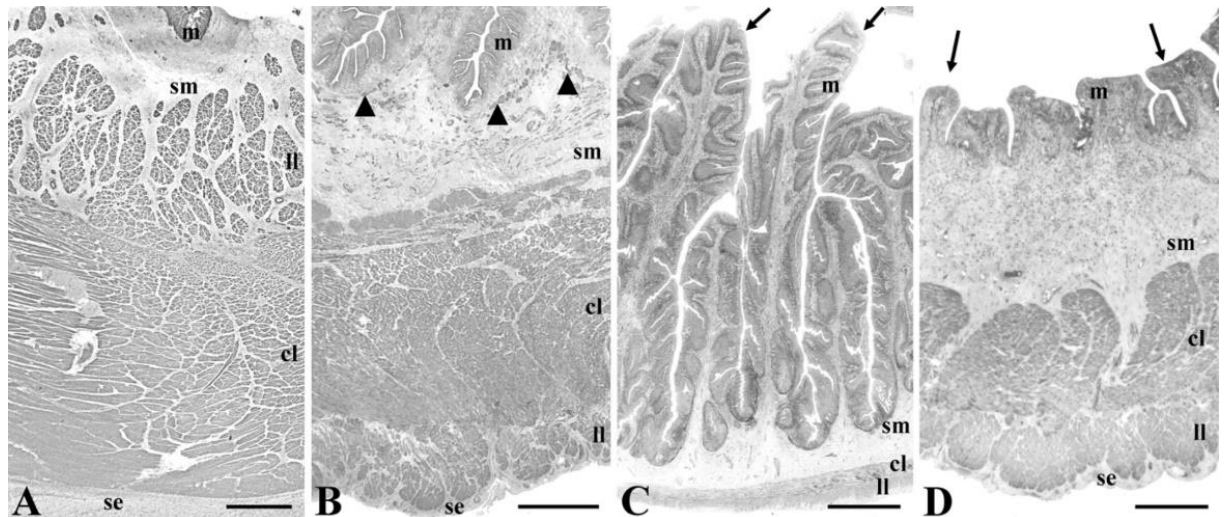


Figure 2. Histological micrographs of different regions of the digestive tract of *H. platyrhynchos* illustrating the general organization: mucosa (m); submucosa (sm); circular layer of muscularis (cl); longitudinal layer of muscularis (ll); serosa (se) (toluidine blue). **(A)** Transversal section of esophagus, note the muscular with an inner longitudinal layer and an outer circular layer. **(B)** Transversal section of stomach showing the muscularis mucosa (arrowheads) and the muscular with an inner circular layer and an outer longitudinal layer. **(C)** Anterior intestine with thick folds (arrows) and thin muscular layer. **(D)** Posterior intestine, with thin folds (arrows) and thick muscular layer. Scale bars: A, B and C= 500 μ m, D= 200 μ m.

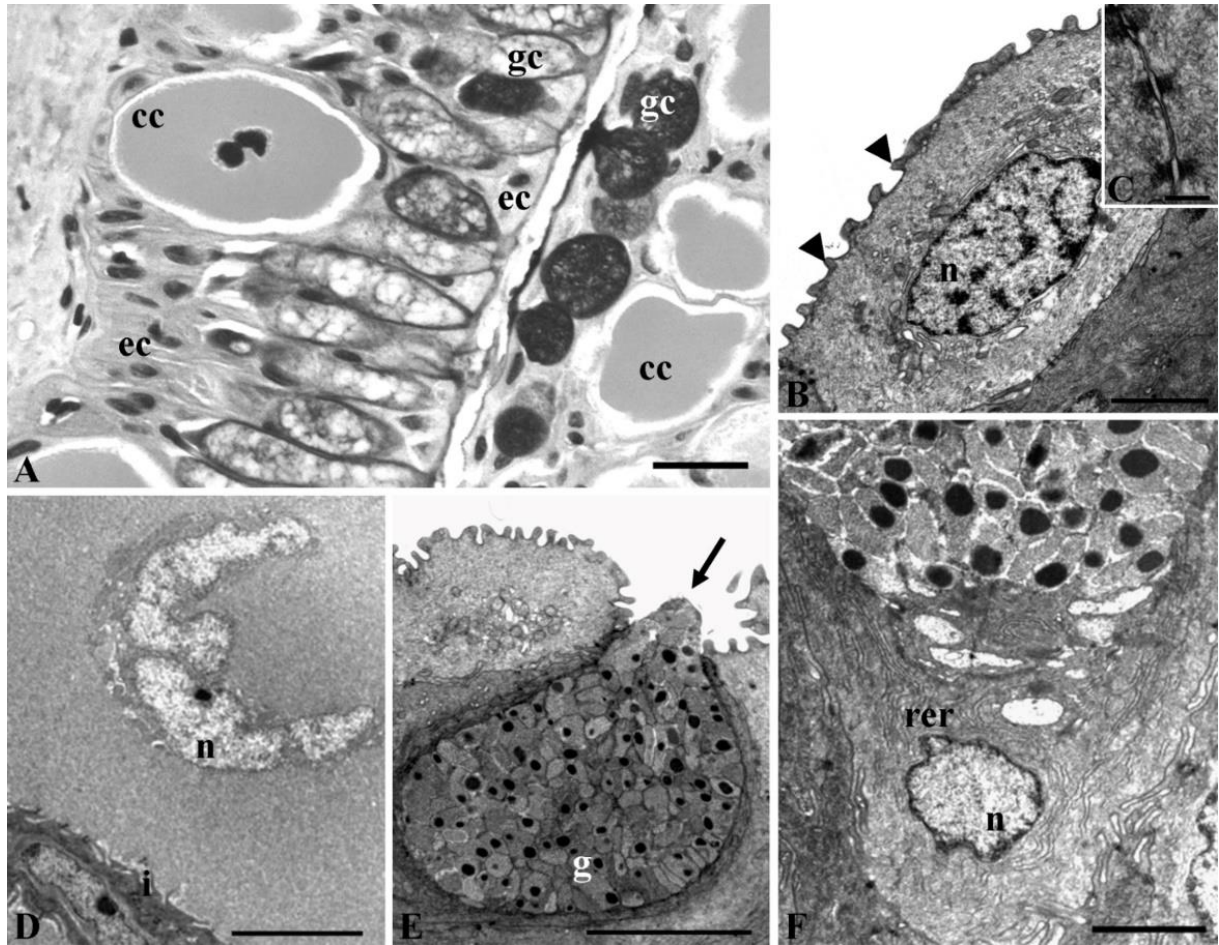


Figure 3. (A) Histological section of esophagus, showing the main cellular types: epithelial cells (ec), club cells (cc) and goblet cells (gc) (hematoxylin and eosin). (B) Electron micrograph of epithelial cell, note the elongated nucleus (n), the fingerprint-like microridges (arrowheads). (C) Desmosomes of epithelial cells. (D) Electron micrograph showing the club cell with irregular nucleus (n) and interdigitations (i). (E) Electron micrograph showing the opening of goblet cell (arrow) and granules (g) of different electrondensity. (F) Goblet cell with a basal and euchromatinic nucleus (n) and rough endoplasmic reticulum (rer). Scale bars: A= 20 μ m, B= 5 μ m, C= 250nm, D= 5 μ m, E= 5 μ m, F= 2 μ m.

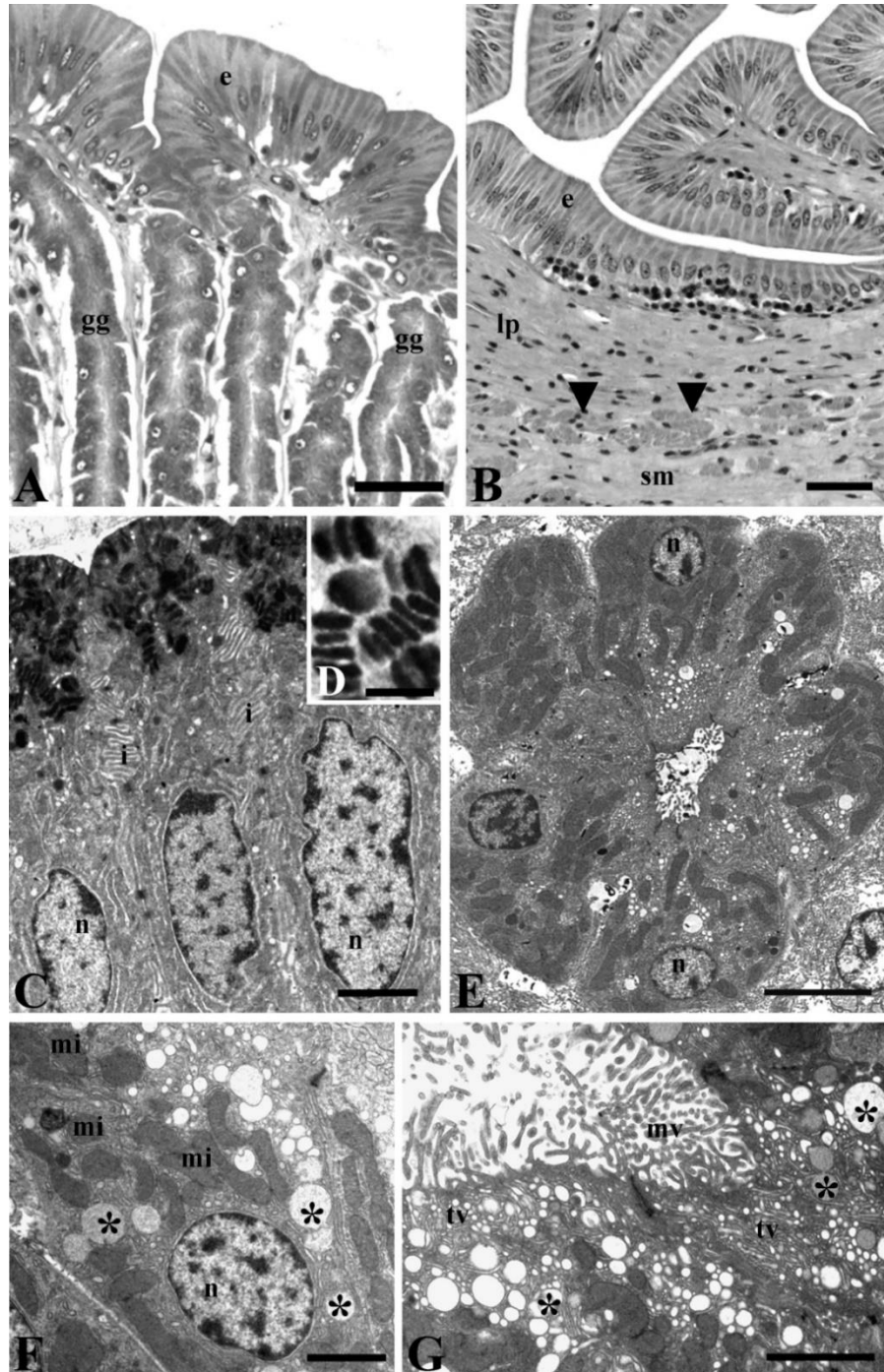


Figure 4. (A) Histological section of the cardiac region of stomach of *H. platyrhynchos*, showing the single columnar epithelium (e) and gastric glands (gg) (toluidine blue). (B) Columnar epithelium (e) of pyloric region of stomach. Note the absence of gastric glands and presence of muscularis mucosa layer (arrowheads), marking the limit of lamina propria (lp) and submucosa (sm) (hematoxylin and eosin). (C) Electron micrograph of epithelial cells, with basal nucleus (n) and interdigitations (i). (D) Mucous granules of epithelial cell, with rod-shaped. (E) Electron micrograph showing the structure of tubular gastric gland, with basal nucleus (n). (F) Oxynticopeptic cell, showing basal nucleus (n), numerous mitochondria (mi) and electron-lucent granules (asterisks). (G) Apical portion of oxynticopeptic cells, with numerous electron-lucent granules (asterisks), tubulovesicular system (tv) and microvilli (mv). Scale bars: A and B= 30 μ m, C= 2 μ m, D= 500nm, E= 5 μ m, F and G= 2 μ m.

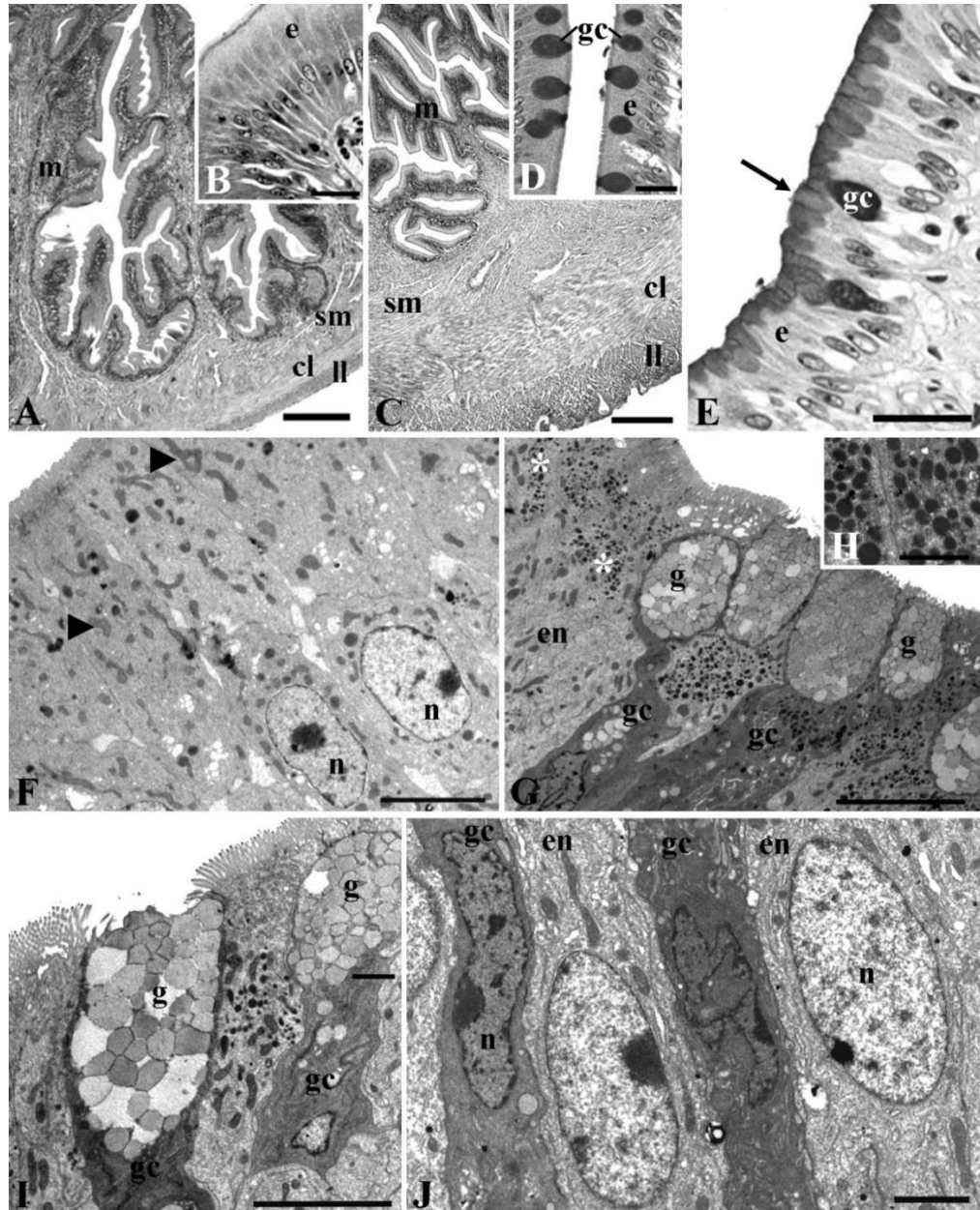


Figure 5. Histological sections of the digestive tract of *H. platyrhynchos*, showing the general organization: mucosa (m), submucosa (sm), inner circular of muscular (cl); outer longitudinal of muscular (ll) (hematoxylin and eosin). (A) Anterior intestine with large folds. (B) Columnar epithelium (e) in anterior intestine. (C) Posterior intestine, note the thick muscular layer. (D) Columnar epithelium (e) with numerous goblet cells (gc) in posterior intestine. (E) Rectum showing simple columnar epithelium (e) with apical mucosubstances (arrow) and scarce goblet cells (gc). (F) Electron micrograph of enterocytes of anterior intestine, with basal nucleus (n) and numerous mitochondria (arrowheads). (G) Posterior intestine, note the numerous secretory granules (g) in goblet cells (gc) and numerous electrondensity spherical lisosomes (asterisks) in enterocytes (en). (H) Lisosomes in enterocytes of posterior intestine. (I) Epithelium of posterior intestine, showing the granules (g) and the opening of goblet cell (gc). (J) Basal region of epithelium in posterior intestine showing the elongated nucleus (n) of enterocytes (en) and irregular nucleus (n) of goblet cell (gc). Scale bar: A=200µm, B=20µm, C=200µm, D and E=20µm, F=5µm, G=10µm, H=2µm, I=5µm, J=2µm.

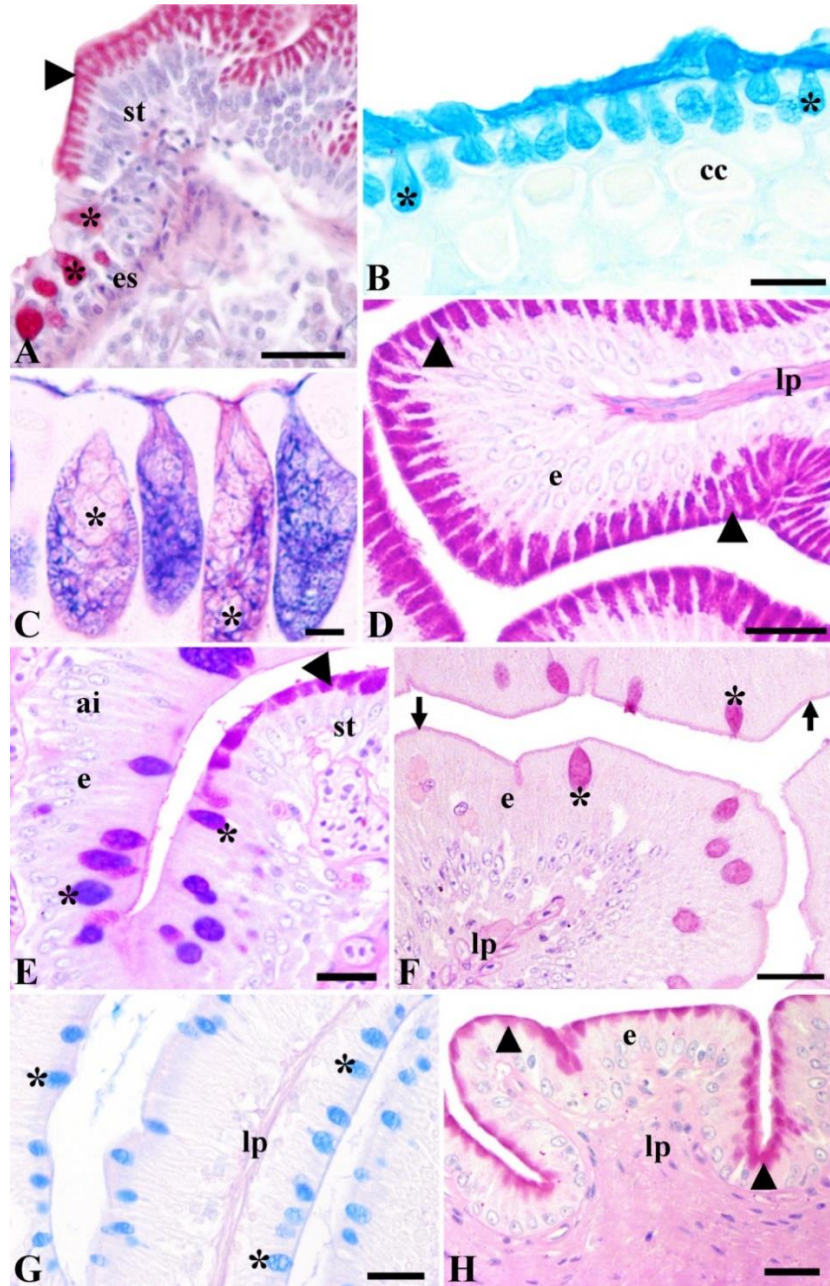


Figure 6. Histochemistry of the digestive tract of the *H. platyrhynchos*. **(A)** Transition esophagus (es) – stomach (st), note the abrupt change of esophageal epithelium with goblet cells (asterisks) to gastric epithelium with epithelial cells with apical mucosubstances (arrowhead) (PAS+hematoxylin). **(B)** Goblet cells (asterisks) AB-positive and club cells (cc) AB-negative in esophagus (AB pH1.0). **(C)** Goblet cells (asterisks) in esophagus show association of acidic and neutral mucosubstances (AB pH2.5+PAS). **(D)** Columnar cells of epithelium of the pyloric region of stomach with apical mucins strongly PAS-positivity (arrowheads). **(E)** Transition stomach (st) – anterior intestine (ai), note the abrupt change of gastric epithelium with apical mucosubstances (arrowhead) to intestinal epithelium with enterocytes and goblet cells (asterisks) (AB pH2.5+PAS+hematoxylin). **(F)** Anterior intestine with brush border (arrows) and goblet cells (asterisks) PAS-positives. **(G)** Posterior intestine with numerous goblet cells (asterisks) (AB pH 2.5). **(H)** Columnar cells of the epithelium of rectum with apical mucins (arrowheads) (PAS+hematoxylin). Legend: e-epithelium; lp-lamina propria. Scale bar: A and B= 30 μ m, C= 6 μ m, D-H= 20 μ m.

Table 1. Histochemical reactions of mucin in the mucosa of the digestive tract of *H. platyrhynchos*.

Techniques employed	Regions									
	Esophagus	Esophagus	Esophagus end portion	Stomach epithelial portion	Stomach glandular portion	Anterior intestine	Midle intestine	Posterior intestine	Rectum	Rectum
PAS	-	+++	+++	+++	+	+++	+++	+++	+++	+++
AB pH 2,5	-	+++	+	+	-	+++	+++	+++	+++	+++
AB pH 1,0	-	+++	-	-	-	+	++	+++	+++	+++
AB (pH 2,5) + PAS	-	++	-	-	-	+	++	++	++	++
	Club cells	Goblet cells	Goblet cells	Epithelial cells	Oxynticopeptic cells	Goblet cells	Goblet cells	Goblet cells	Epithelial cells	Goblet cells

Staining intensity: (-) negative; (+) weak; (++) moderate; (+++) strong.

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Ultrastructure of the digestive tract in Neotropical carnivorous catfish *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Siluriformes, Pimelodidae)

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Summary

The surface of the digestive tract of *Hemisorubim platyrhynchos* was analyzed by scanning electron microscopy. Morphometric studies by transmission electron microscopy were performed to analysis the intestinal microvilli. *H. platyrhynchos* is a Neotropical carnivorous freshwater catfish featuring a short digestive tract composed of a short esophagus, saccular stomach and intestine with four regions: anterior, middle, posterior and rectal. The esophageal surface is constituted by fingerprint-like microridges that anchor the mucosubstances secreted by goblet cells facilitating the passage of food. Goblet cells present the opening to the esophageal lumen, between the microridges. Club cells are in basal epithelium and they do not present the opening to the lumen. The gastric luminal surface shows polygon-shaped epithelial cells which secrete granules by exocytose to protect the gastric surface. The intestinal luminal surface reveals folds that are thicker in the anterior intestine than in the posterior intestine, increasing the absorptive surface area. The intestinal surface presents the microvilli of enterocytes and the opening of goblet cells. The morphometric analysis showed that the microvilli are longer in the anterior intestine, significantly decreasing towards the posterior intestine. The microvilli surface area significantly is greater in the anterior and middle intestine than in the posterior intestine. Numerous openings of goblet cells were observed in the posterior intestine acting in epithelial protection and lubrication.

Keywords: surface analysis, morphometry, microvilli, *Hemisorubim platyrhynchos*.

3.2.1 Introduction

The digestive tract of teleosts is generally composed of an esophagus, a stomach, pyloric caeca and an intestine (Hossain and Dutta, 1996). However, variations such as the presence or absence of stomach and pyloric caeca, length and shape of organs, pattern of folds and epithelial specializations, have been reported. Several studies have used scanning electron microscope to describe the morphology of the digestive tract of teleosts to comprehend the function of specialized structures of each organ in relation to different feeding adaptations (Humbert *et al.*, 1984; Grau *et al.*, 1992; Soares, *et al.*, 1995; Mir and Channa, 2010; Namulawa *et al.*, 2013). Another analysis, which has been used by researchers, is the morphometry of the microvilli, allowing infer about of the absorption of nutrients in fish (German *et al.*, 2010, Zeng *et al.*, 2014). Although there is an increasing interest in the morphological aspects of the digestive system of fish, such studies are still scarce for Neotropical ichthyofauna (Canan *et al.*, 2012), which hinders the understanding of the function of each region of digestive tract.

Hemisorubim platyrhynchos, popularly called “jurupoca”, belongs to the family Pimelodidae and the order Siluriformes. It is a migratory species, without parental care, widely distributed throughout the Neotropical region, with reports for the Orinoco, Amazon, Paraguay, Uruguay and Paraná River basins. According to Bressan *et al.* (2009), *H. platyrhynchos* is a nocturnal carnivorous fish. Like others Pimelodidae, *H. platyrhynchos* is predator of others fish and is therefore important for the functioning of aquatic ecosystems (Penha and Mateus, 2007). In addition, there has been a reduction in the population size of the species due to habitat destruction as a result of the construction of hydroelectric dams that interrupt the flow of migration required for reproduction (Bressan *et al.*, 2009). The species is valuable for aquaculture because of the quality and flavor of its meat and the absence of intramuscular bones. Moreover, the total catch of *H. platyrhynchos* was 50,0 tons in upper Paraguai River Basin in Mato Grosso do Sul State, between 1994 and 1999 (Catella, 2001). Thus, to complement previous work performed by Faccioli *et al.* (2014), this study aimed to describe the digestive tract of *H. platyrhynchos* using scanning electron microscopy and to measure the intestinal microvilli by transmission electron microscopy, focusing on the morphology of the epithelial layer to better understand their surface adaptations for a diet based on fish as well as to infer about the site of nutrient absorption.

3.2.1 Material and Methods

3.2.2.1 Animals

Twenty adult specimens of *H. platyrhynchos* were obtained from Pirai Pisciculture (Terenos MS Brazil). Total body length of the specimens was 35.2 ± 2.3 cm. The animals were kept fasting in tanks for three days to empty the digestive tract. After this period, they were anesthetized, euthanized with an overdose of benzocaine and dissected with a longitudinal incision along the ventral region.

3.2.2.2 Scanning Electron Microscopy

Fragments of esophagus, stomach and intestine were fixed for 24 hours at 4°C in a solution of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4). The samples were post-fixed for 2 hours in 1% osmium tetroxide (pH 7.4) and dehydrated in a graded ethanol series. These fragments were processed to obtain the critical point (EMS 850, Electron Microscopy Sciences, USA) and sputtered with colloidal gold vacuum Desk II (Denton Vacuum, USA) (Muñoz *et al.*, 2011). Analysis and photographic documentation were

performed with scanning electron microscope Quanta 200 (Fei, USA) at the Electron Microscopy Center of the Institute of Biosciences (UNESP) in Botucatu SP Brazil.

3.2.2.3 Morphometric Analyses by Transmission Electron Microscopy

Fragments of the intestine were fixed for 24 hours at 4°C in a solution of 4% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4) to analyze the morphometry of intestinal microvilli. Samples were then post-fixed for 2 hours in 1% osmium tetroxide (pH 7.4), dehydrated in a graded acetone series and embedded in Araldite resin. Ultra-thin sections (60 and 80 nm) were mounted on copper grids and contrasted with uranyl acetate and lead citrate. Analysis and photographic documentation were performed with a Philips CM 100 transmission electron microscope (Netherlands). Images of intestinal epithelium (n=10 per intestinal region) were used for measurements of height (H) and diameter (D) of the microvilli (15–70 microvilli per image), using the program Image Pro-Plus 4.1 (Media Cybernetics, USA). With the above data, the microvilli surface area was calculated, such as MVSA (μm^2) = $(H\pi D) + (\pi R^2)$, where $R=0.5D$ (Frierson and Foltz, 1992; German *et al.*, 2010; Zeng *et al.*, 2014).

3.2.2.4 Statistical Analyses

Height, diameter and surface area of microvilli among the different regions of intestine were compared with ANOVA, followed by Tukey's HSD with a familywise error rate of $P<0.05$.

3.2.2.5 Ethical Note

Current study was approved by the Committee for Ethics in Research of the Faculty of Sciences of the São Paulo State University (UNESP), Bauru SP Brazil, under protocol n. 1144/46/01/10.

3.2.3 Results

The luminal surface of the digestive tract of *H. platyrhynchos* showed differences among the organs, both in relation to the folds pattern and to cellular surface specializations. The esophagus is a short tubular organ located dorsally to the liver. This organ has a thick wall and longitudinal folds in luminal surface (Fig. 1A). The lining epithelium of the esophagus is

stratified, with three main cellular types: epithelial cells, goblet cells and club cells. Taste buds were not found in the esophageal epithelium.

Epithelial cells formed a continuous apical layer throughout the esophageal epithelium. The surface of these cells presents fingerprint-like microridges formed from the apical plasma membrane (Fig. 1B-1F). The microridges show a whorled, usually unbranched, pattern. These structures dominate the surface of the epithelium and are interrupted only by the opening of the goblet cells (Fig. 1B-1F).

Goblet cells are concentrated in the apical region of the epithelium forming a single layer of cells with the opening to the esophageal lumen (Fig. 1C and 1D). Esophageal goblet cells have different shapes: oval, elongated or cup (Fig. 1C-1F), and are filled with secretory granules that occupy most of the cytoplasm (Fig. 1E and 1F). The other esophageal cellular type, the club cells, appears in the basal region of the epithelium but they are scarce in the final portion of the esophagus (Fig. 1C and 1D). These voluminous cells are elongated or round. Unlike the goblet cells, club cells show no opening to the esophageal lumen (Fig. 1D).

The stomach is a saccular organ with a thick wall that consists of the cardiac, fundic and pyloric regions. Folds were detected throughout the gastric lumen and in the fundic region they present different orientations (Fig. 2A). The stomach shows simple epithelium constituted by columnar epithelial cells (Fig. 2B). The surface of epithelial cells has a polyhedron shape, predominantly hexagonal (Fig. 2C and 2D). Moreover, in the surface of these cells were observed granules secretion (Fig. 2D).

The intestine consists of the anterior, middle, posterior and rectal regions. Pyloric caeca were not observed in the intestine of *H. platyrrhynchos*. The anterior intestine has a thin wall and thick primary longitudinal folds, whereas the posterior intestine has a thick wall and thin primary longitudinal folds (Fig. 3A and 3C). The primary folds present a central axis and secondary folds, formed from the central axis of primary fold, were found in anterior intestine (Fig. 3A and 3B). The lining of the intestinal epithelium mainly consists of enterocytes and goblet cells. The luminal surface of the intestine shows numerous microvilli and the openings of goblet cells. The openings of goblet cells are more numerous in the posterior intestine (Fig. 3D-3F). The morphometric analysis by transmission electron microscopy showed that the microvilli are longer in the anterior intestine, significantly decreasing towards the posterior intestine (ANOVA $F=379.1$, $P<0.01$; Fig. 4). The diameter of microvilli was not varied among the intestinal regions (ANOVA $F=0.5032$, $P>0.6$). The microvilli surface area was greater in

the anterior intestine ($0.298 \pm 0.048 \mu\text{m}^2$) and middle intestine ($0.285 \pm 0.034 \mu\text{m}^2$) than in the posterior intestine ($0.182 \pm 0.020 \mu\text{m}^2$), but significantly greater in the anterior and middle intestine than in the posterior intestine (ANOVA $F=17.68$, $P<0.0001$). The morphometric data are summarized in Figure 5.

3.2.4 Discussion

This study describes in detail the surface of the digestive tract in freshwater carnivorous catfish *H. platyrhynchos*. According to Faccioli *et al.* (2014), the species presents a short digestive tract with a tubular esophagus, prominent stomach and few convoluted intestine. The short gut is a characteristic feature of carnivorous teleosts (Ezeasor and Stokoe, 1980). Hossain and Dutta (1996) estimated that 60% of known fish species possess structures, such as pyloric caeca, related to a greater surface area for digestion and absorption. However, *H. platyrhynchos* lacks this organ, similar to other Siluriformes as *Ictalurus punctatus* (Sis *et al.*, 1979), *Pelteobagrus fulvidraco* (Cao and Wang, 2009), *Rhamdia quelen* (Hernández *et al.*, 2009), *Claris batrachus* (Raji and Norouzi, 2010). According to Carrassón and Matallanas (1994), the pyloric caeca are related to phylogeny rather than a feature associated to diet.

The esophagus is a short organ with thick longitudinal folds, which enables the distention of the organ to swallow the prey (Park and Kim, 2001; Rodrigues and Menin 2008). On the other hand, the esophagus of *H. platyrhynchos* has no taste buds which are structures that play a significant role in the selection, sorting out, and retention of palatable food particles ingested by the fish (Yashpal *et al.*, 2009; Elsheikh *et al.*, 2012). Thus, the esophagus of the studied species has no role in the selection of food; rather it directs the food from the oropharyngeal cavity to the stomach (Ezeasor and Stokoe, 1980). The esophagus of *H. platyrhynchos* shows a stratified epithelium which mainly consists of epithelial cells, goblet cells and club cells (Faccioli *et al.*, 2014). To facilitate the passage of food, the surface of esophageal epithelial cells shows fingerprint-like microridges. These structures also have been described in several teleosts, such as *Salmo gairdneri* (Sperry and Wassersug, 1976), *Pseudoplatystoma corruscans* (Soares *et al.*, 1995), *Solea senegalensis* (Arellano *et al.*, 2001), *Dentex dentex* (Carrassón *et al.*, 2006), *Lates niloticus* (Namulawa *et al.*, 2013). According to Uehara *et al.* (1990), the microridges are formed by actin filaments anchored to aggregated keratin filaments and they appear in fish organs that are subject to mechanical stress (Sinha and Chakrabarti, 1986). The microridges may therefore represent an epithelial protection against mechanical injury and also anchor the mucins secreted by goblet cells, thereby forming a

lubricated epithelial surface for the passage of food (Sperry and Wassersug, 1976; Ezeasor and Stokoe, 1980; Humbert *et al.*, 1984; Grau *et al.*, 1992; Abaurrea-Equisoain and Ostos-Garrido, 1996, Wilson and Castro, 2010).

Goblet cells are abundant and form a continuous cellular layer in the esophageal epithelium of *H. platyrhynchos*. According to Scocco *et al.* (1998), the large amount of mucosubstances produced in the esophagus may be explained by the absence of salivary glands in fish. The mucosubstances reduce friction to facilitate the swallowing of the prey (Namulawa *et al.*, 2013). Thus, the mucosubstances secreted by goblet cells may protect the epithelium throughout the digestive tract facilitating the passage of food. The esophageal epithelium of *H. platyrhynchos* also presents numerous club cells, especially in the anterior region of the esophagus (Faccioli *et al.*, 2014). These cells have no openings to the esophageal lumen. According to Nakamura *et al.* (2001), the club cells of *Conger myriaster* present only protein secretion and display holocrine secretion via rupture of the cell during epithelial injury, forming the first line of defense to protect the tissues underlying the epithelium (Chivers *et al.*, 2007).

The stomach of *H. platyrhynchos* is divided into cardiac, fundic and pyloric regions. Folds with different orientations were observed in the luminal surface of the fundic region, which probably enable the distension of the stomach during ingestion of large prey and also increase the surface area for digestive activity (Sinha and Chakrabarti, 1986; Namulawa *et al.*, 2013). The gastric surface of *H. platyrhynchos* presents polyhedron-shaped cells, similar to those observed in other teleosts such as *Anguilla anguilla* that has a pentagonal mosaic pattern (Clarke and Witcomb, 1980), *Salmo gairdneri* that has square, pentagon or hexagon cells (Ezeasor and Stokoe, 1980), *Gymnothorax pictus* (Takiue and Akiyoshi, 2013) and *Lates niloticus* (Namulawa *et al.*, 2013) that has polygon-shaped cells. Moreover, the surface of epithelial cells of the studied species shows secretion granules. These features have also been found in histochemical studies of the gastric mucosa of *H. platyrhynchos* (Faccioli *et al.*, 2014). According to Naguib *et al.* (2011), the granules of epithelial cells of the stomach of *Schilbe mystus* appear frequently in contact with the apical plasma membrane to empty its contents into the gastric lumen. These granules are secreted into the gastric lumen by exocytosis (Noaillac-Depeyre and Gas, 1978; Ostos-Garrido *et al.*, 1993) and they protect the epithelium of stomach from autodigestion processes caused by secretions produced in the gastric glands (Ferraris *et al.*, 1987). Other authors found microvilli related to absorption in the gastric epithelial cells in some teleosts, such as *Salmo gairdneri* (Ezeasor and Stokoe, 1980), *Mystus aor* (Sinha and

Chakrabarti, 1986), *Gymnothorax pictus* (Takiue and Akiyoshi, 2013). However, the surface of stomach of *H. platyrrhynchus* has no microvilli, which highlights the primary function of the stomach in food digestion.

The intestine of *H. platyrrhynchus* presents developed folds in the anterior region. Intestinal folds are associated with the retention time of food (Cao and Wang, 2009) and also with the increase of the surface area for absorption (Løkka *et al.*, 2013). The intestine of *H. platyrrhynchus* is lined by a columnar epithelium composed mainly of enterocytes and goblet cells. The luminal intestinal surface shows numerous microvilli which increase the surface area for absorption (Wilson and Castro, 2010; Løkka *et al.*, 2013). Microvilli of *H. platyrrhynchus* are longer in the anterior intestine, significantly decreasing towards the posterior intestine. On the other hand, the microvilli diameter is not varied among intestinal regions and, according to Zeng *et al.* (2014), the measurement of the microvilli surface area depends mainly on the microvilli height. The microvilli surface area of *H. platyrrhynchus* was statistically greater in anterior and middle intestine than in the posterior intestine. Frierson and Foltz (1992) concluded that in the case of *Tilapia aurea* and *Tilapia zilli*, microvilli accounted for more than 90% of the digestive surface area and the anterior intestine of teleosts has been considered the main site of nutrient absorption (Radaelli *et al.*, 2000; Løkka *et al.*, 2013). In this sense, the thick folds and the long microvilli of the anterior intestine of *H. platyrrhynchus* indicate an increase in the retention time of food and in the surface area to complete digestion. Thus, these features suggest that the anterior intestine is the main site of absorption.

On the other hand, the surface of the posterior intestine of *H. platyrrhynchus* shows more openings of the goblet cells than those in the anterior intestine. Similar results were observed in *Misgurnus mizolepis* (Park and Kim, 2001) and *Anguilla anguilla* (Domeneghini *et al.*, 2005). In turn, Sinha and Chakrabarti (1986) observed few goblet cells in the intestine of *Mystus aor* and the authors emphasize that carnivorous fish do not require much mucins for gluing the ingested food. This would explain the low amount of goblet cells in anterior intestine of *H. platyrrhynchus*, whereas in the posterior intestine, the great number of goblet cells probably acts as epithelial protection and lubrication. According to Carrassón *et al.* (2006), the secretions of goblet cells of *Dentex dentex* are thought to provide protection for the epithelium and lubrication to facilitate the transit of food.

This study provided more in-depth information on the morphofunctional aspects of the digestive tract in carnivorous fish. *H. platyrrhynchus* features specializations as folds with

different pattern throughout the digestive tract that are associated to the function of each organ. The fingerprint-like microridges protect the surface of esophagus against mechanical injury and also anchor the mucins to facilitate food swallow. The microvilli of the intestine, mainly in the anterior intestine, increase the surface area for digestion and absorption. These surface specializations enable the *H. platyrhynchos* ingest, digest and absorb food, since it is a carnivorous catfish that capture large fish.

3.2.5 Acknowledgements

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3.2.6 References

- Abaurrea-Equisoain MA, Ostos-Garrido MV. 1996. Cell types in the esophageal epithelium of *Anguilla anguilla* (Pisces, Teleostei). Cytochemical and Ultrastructural Characteristics. *Micron* 27:419-429.
- Arellano JM, Storch V, Sarasquete C. 2001. A histological and histochemical study of the oesophagus and oesogaster of the Senegal sole, *Solea senegalensis*. *Eur J Histochem* 45:279-294.
- Bressan PM, Kierulff MCM, Sugieda AM. 2009. Fauna ameaçada de extinção no estado de São Paulo: Vertebrados. Fundação Parque Zoológico de São Paulo: Secretaria do Meio Ambiente.
- Cao XJ, Wang WM. 2009. Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. *Anat Histol Embryol* 38:254-261.
- Canan B, Nascimento WS, Silva NB, Chellappa S. 2012. Morphohistology of the Digestive Tract of the Damsel Fish *Stegastes fuscus* (Osteichthyes: Pomacentridae). *Sci World J* 2012:1-9.
- Carrassón M, Matallanas J. 1994. Morphometric characteristics of the alimentary tract of deep-sea Mediterranean teleosts in relation to their feeding habits. *Mar Biol* 118:319-322.
- Carrassón M, Grau A, Dopazo LR, Crespo S. 2006. A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). *Histol Histopathol* 21:579-593.
- Catella, AC. 2001. A pesca no Pantanal de Mato Grosso do Sul, Brasil: Descrição, nível de exploração e manejo (1994 - 1999). PhD Thesis, 351p. Instituto Nacional de Pesquisas da Amazônia- INPA, Manaus.

- Chivers DP, et al. 2007. Epidermal 'alarm substance' cells of fishes maintained by non-alarm functions: possible defence against pathogens, parasites and UVB radiation. *P Roy Soc B* 274:2611-2619.
- Clarke AJ, Witcomb DM. 1980. A study of the histology and morphology of the digestive tract of the common eel (*Anguilla anguilla*). *J Fish Biol* 16:159-170.
- Domeneghini C, Arrighi S, Radaelli G, Bosi G, Veggetti A. 2005. Histochemical analysis of glycoconjugates secretion in the alimentary canal of *Anguilla anguilla* L. *Acta Histochem* 106:477-487.
- Elsheikh EH, Nasr ES, Gamal AM. 2012. Ultrastructure and distribution of the taste buds in the buccal cavity in relation to the food and feeding habit of a herbivorous fish: *Oreochromis niloticus*. *Tissue Cell* 44:164-169.
- Ezeasor DN, Stokoe WM. 1980. Scanning electron microscopic study of the gut mucosa of the rainbow trout *Salmo gairdneri* Richardson. *J Fish Biol* 17:529-539.
- Faccioli CK, Chedid RA, Amaral AC, Franceschini-Vicentini IB, Vicentini CA. 2014. Morphology and histochemistry of the digestive tract in carnivorous freshwater *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae). *Micron* 64: 10–9.
- Ferraris RP, Tan JD, De la Cruz MC. 1987. Development of the digestive tract of milkfish, *Chanos chanos* (Forsskal): Histology and histochemistry. *Aquaculture* 61:241-257.
- Frierson EW, Foltz JW. 1992. Comparison and estimation of absorptive intestinal surface areas in two species of cichlid fish. *T Am Fish Soc* 121:517-523.
- German DP, Neuberger DT, Callahan MN, Lizardo NR, Evans DH. 2010. Feast to famine: The effects of food quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae). *Comp Biochem Physiol A* 155:281-293.
- Grau A, Crespo S, Sarasquete MC, Gonzales de Canales ML, 1992. The digestive tract of the amberjack *Seriola dumerili* Risso: a light and scanning electron microscope study. *J Fish Biol* 41:287-303.
- Hernández DR, Pérez Ganeselli M, Domitrovic HA. 2009. Morphology, histology and histochemistry of the digestive system of south american catfish (*Rhamdia quelen*). *Int J Morphol* 27:105-111.
- Hossain AM, Dutta HM. 1996. Phylogeny, ontogeny, structure and function of digestive tract appendages (caeca) in teleost fish. In: Munshi JSD, Dutta HM, eds. *Fish morphology horizon of new research*. Brookfield: A.A Balkema Publishers.
- Humbert W, Kirsch R, Meister MF. 1984. Scanning electron microscopic study of the oesophageal mucous layer in the eel *Anguilla anguilla* L. *J Fish Biol* 25:117-122.
- Løkka G, Austbø L, Falk K, Bjerkå I, Koppang EO. 2013. Intestinal morphology of the wild atlantic salmon (*Salmo salar*). *J Morphol* 274:859-76.
- Mir IH, Channa A. 2010. Histochemical distribution of lipase and acid phosphatase in the intestinal tract of the snow trout, *Schizothorax curvifrons* Heckel. *J Biol Sci* 10:643-647.
- Muñoz ME, Batlouni SR, Franceschini-Vicentini IB, Vicentini CA. 2011. Testicular structure and description of the seminal pathway in *Leporinus macrocephalus* (Anostomidae, Teleostei). *Micron* 42:892–897

- Naguib SAA, Ei-Shabaka HA, Ashour F. 2011. Comparative histological and ultrastructural studies on the stomach of *Schilbe mystus* and the intestinal swelling of *Labeo niloticus*. J Am Sci 7:251-263.
- Nakamura O, Watanabe T, Kamiya H, Muramoto K. 2001. Galectin containing cells in the skin and mucosal tissues in Japanese conger eel, *Conger myriaster*: an immunohistochemical study. Dev Comp Immunol 25:431-437.
- Namulawa VT, Kato CD, Nyatia E, Rutaisire J, Britz PJ. 2013. Scanning electron microscopy of the gastrointestinal tract of Nile perch (*Lates niloticus*, Linnaeus, 1758). Int J Morphol 3:1068-1075.
- Noaillac-Depeyre J, Gas N, 1978. Ultrastructural and cytochemical study of the gastric epithelium in a fresh water teleostean fish *Perea fluviatilis*. Tissue Cell 10:23-37.
- Ostos-Garrido MV, Gonzalez-Oller C, Abaurrea-Equisoain MA. 1993. Histological, histochemical and ultrastructural analysis of the gastric mucosa in *Oncorhynchus mykiss*. Aquaculture 115:121-132.
- Park JY, Kim IS. 2001. Histology and mucin histochemistry of the gastrointestinal tract of the mud loach, in relation to respiration. J Fish Biol 58:861-872.
- Penha JMF, Mateus LAF. 2007. Sustainable harvest of two large predatory Catfish in the Cuiabá river basin, northern Pantanal, Brazil. Braz J Biol 67:81-89.
- Radaelli G, Domeneghini C, Arrighi S, Francolini M, Mascarello F. 2000. Ultrastructural features of the gut in the white sturgeon, *Acipenser transmontanus*. Histol Histopathol 15:429-439.
- Raji AR, Norouzi E. 2010. Histological and histochemical study on the alimentary canal in Walking catfish (*Claris batrachus*) and piranha (*Serrasalmus nattereri*). Iran J Vet Res 11:255-261.
- Rodrigues SS, Menin E. 2008. Anatomia do tubo digestivo de *Salminus brasiliensis* (Cuvier, 1817) (Pisces, Characidae, Salmininae). Biotemas 21:65-75.
- Scocco P, Accili D, Menghi G, Ceccarelli P. 1998. Unusual glycoconjugates in the esophagus of a tilapine polyhybrid. J Fish Biol 53:39-48.
- Sinha GM, Chakrabarti P. 1986. Scanning electron microscopic studies on the mucosa of the digestive tract in *Mystus aor* (Hamilton). P Indian Natl Sci Acad B, 52, 267-273.
- Sis RF, Ives PJ, Jones DM, Lewis DH, Haensly WE. 1979. The microscopic anatomy of the oesophagus, stomach and intestine of the channel catfish, *Ictalurus punctatus*. J Fish Biol 14:179-186.
- Soares TRS, Watanabe IS, Lopes RA, Semprini M, Bahls AS, Brentegani LG, Satake T. 1995. Light and scanning electron microscopic study of the digestive tract in the catfish *Pseudoplatystoma corruscans* Agassiz, 1829 (Pisces, Pimelodidae). Braz J Morphol Sci 12:101-108.
- Sperry DG, Wassersug RJ. 1976. A proposed function for microridges on epithelial cells. Anat Rec 185:253-257.
- Takiue S, Akiyoshi H. 2013. Light and scanning electron microscope examination of the digestive tract in peppered moray eel, *Gymnothorax pictus* (Elopomorpha). Anat Rec 296:443-451.

- Uehara K, Miyoshi M, Miyoshi S. 1990. Actin filaments in microridges of the oral mucosal epithelium in the carp *Cyprinus carpio*. *Cell Tissue Res* 261:419-422.
- Wilson JM, Castro LFC. 2010. Morphological diversity of the gastrointestinal tract in fishes. In: Grosell M, Farrell AP, Brauner CJ eds. *Fish physiology: the multifunctional gut of fish*. San Diego: Academic Press.
- Yashpal M, Kumari U, Mittal S, Mittal AK. 2009. Morphological specialization of the buccal cavity in relation to the food and feeding habit of a carp *Cirrhinus mrigala*: a scanning electron microscopic investigation. *J Morphol* 270:714-728.
- Zeng LQ, Fu SJ, Li XM, Li FJ, Li B, Cao ZD, Zhang YG. 2014. Physiological and morphological responses to the first bout of refeeding in southern catfish (*Silurus meridionalis*). *J Comp Physiol B* 184:329–346.

3.2.7 Figure Legends

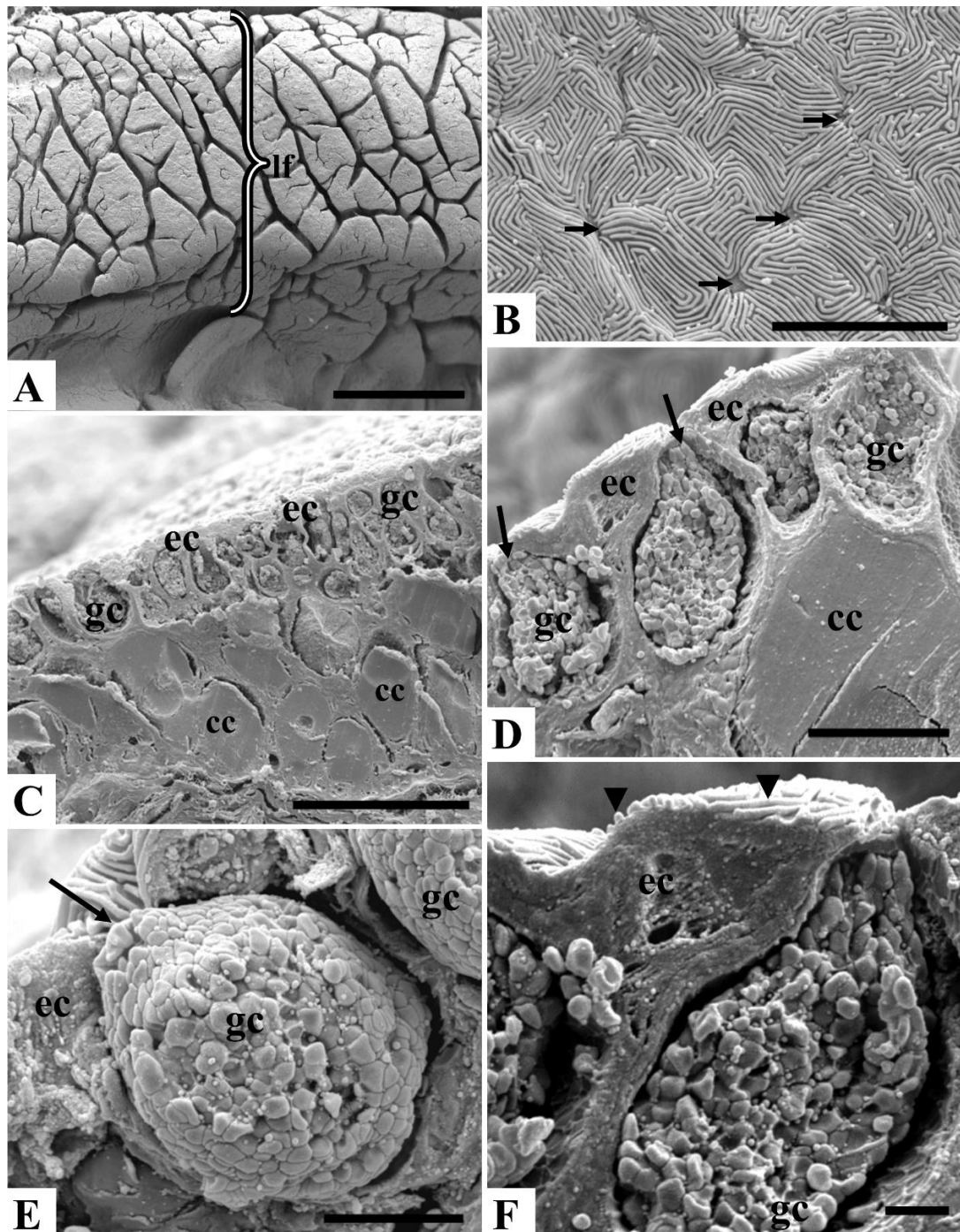


Figure 1. Scanning electron micrographs of the esophagus of *Hemisorubim platyrhynchos*. (A) Esophageal surface showing the longitudinal fold. (B) Surface of esophageal epithelium with fingerprint-like microridges of epithelial cells and opening of goblet cells. (C) Fingerprints-like microridges of esophageal epithelium. (D and E) Scanning electron microscopy of longitudinal section of stratified squamous epithelium, showing epithelial cells, goblet cells and club cells. (F) Goblet cells in apical region of epithelium, evidencing the opening of goblet cells between the epithelial cells. Legends: lf-longitudinal fold, ec-epithelial cell, gc-goblet cell, cc-club cell, opening of goblet cells-arrow, microridge-arrowhead. Scale bar: A. 500µm, B. 10µm, C. 2µm, D. 50µm, E. 10µm, F. 5µm.

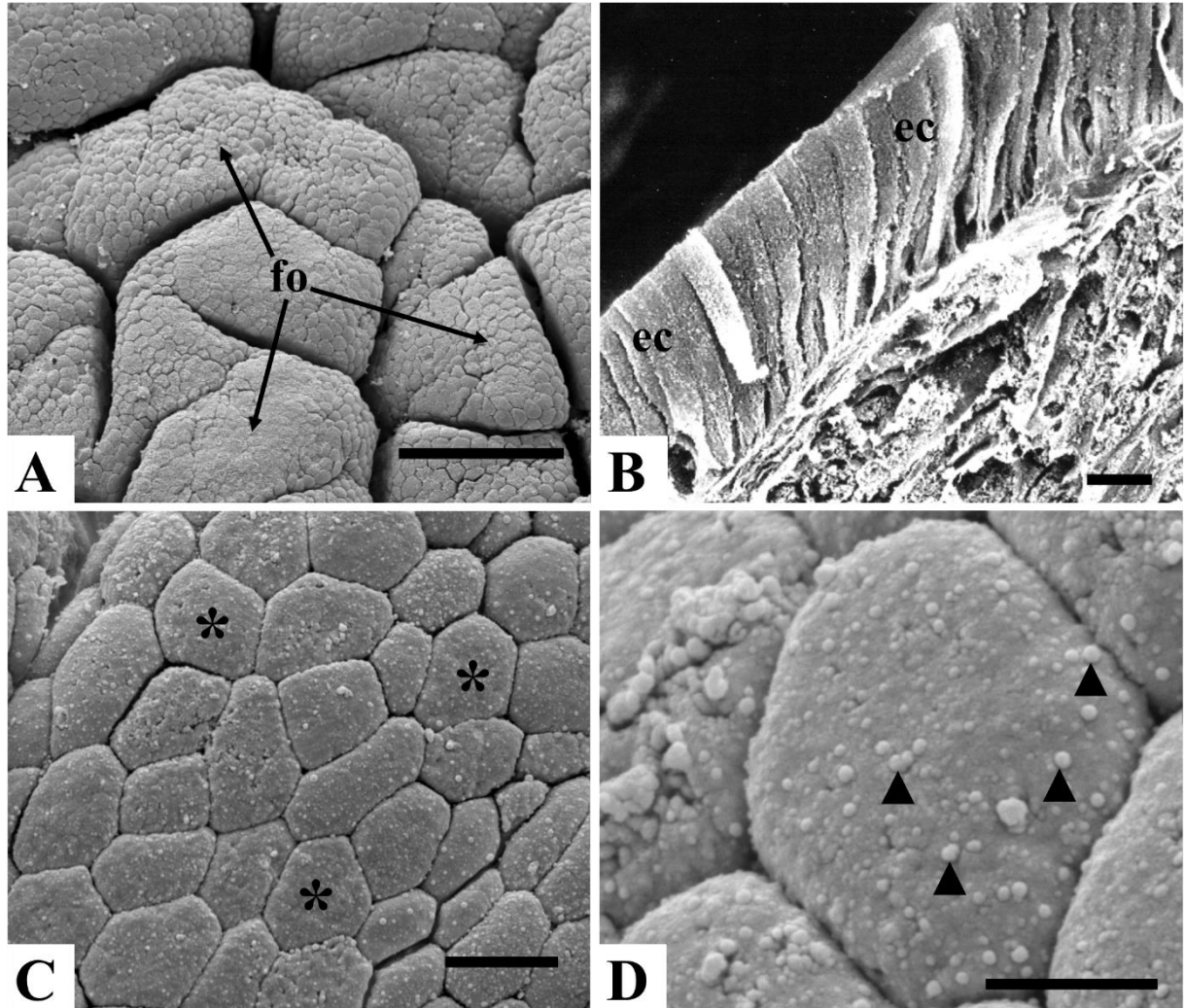


Figure 2. Scanning electron micrographs of the stomach of *Hemisorubim platyrhynchos*. (A) Luminal surface in fundic region of stomach, showing folds in different orientations. (B) Longitudinal section of simple epithelium, evidencing columnar epithelial cells. (C and D) Surface of gastric epithelium showing the polygonal shape of epithelial cells and the secretion granules. Legends: fo-folds, ec-epithelial cell, surface of epithelial cell-asterisk, granules-arrowhead. Scale bar: A. 50 μ m, B. 10 μ m, C. 5 μ m, D. 2 μ m.

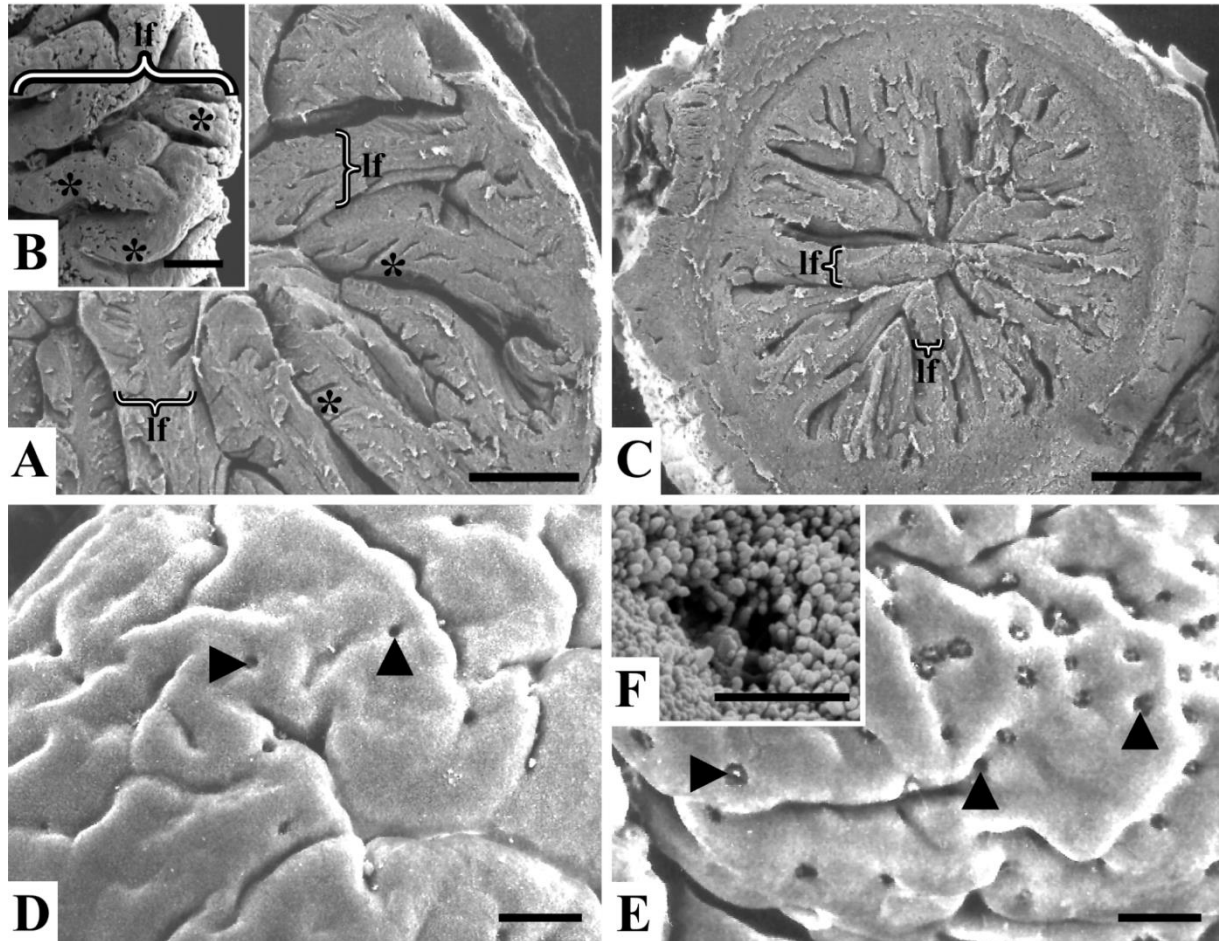


Figure 3. Scanning electron micrographs of the intestine of *Hemisorubim platyrhynchos*. (A) Transversal section of the anterior intestine, evidencing thick primary longitudinal folds and secondary folds. (B) Surface of primary fold, evidencing the secondary folds. (C) Transversal section of posterior intestine, showing thin longitudinal folds. (D and E) Luminal surface of intestine, showing that the opening of goblet cells are more numerous anterior intestine (D) than in posterior intestine (E). (F) Surface of posterior intestine, evidencing the opening of goblet cell. Legends: lf-primary fold, secondary fold-asterisk, opening of goblet cell-arrowhead. Scale bar: A. 500µm, B. 100µm, C. 500µm, D and E. 10µm, F. 2µm.

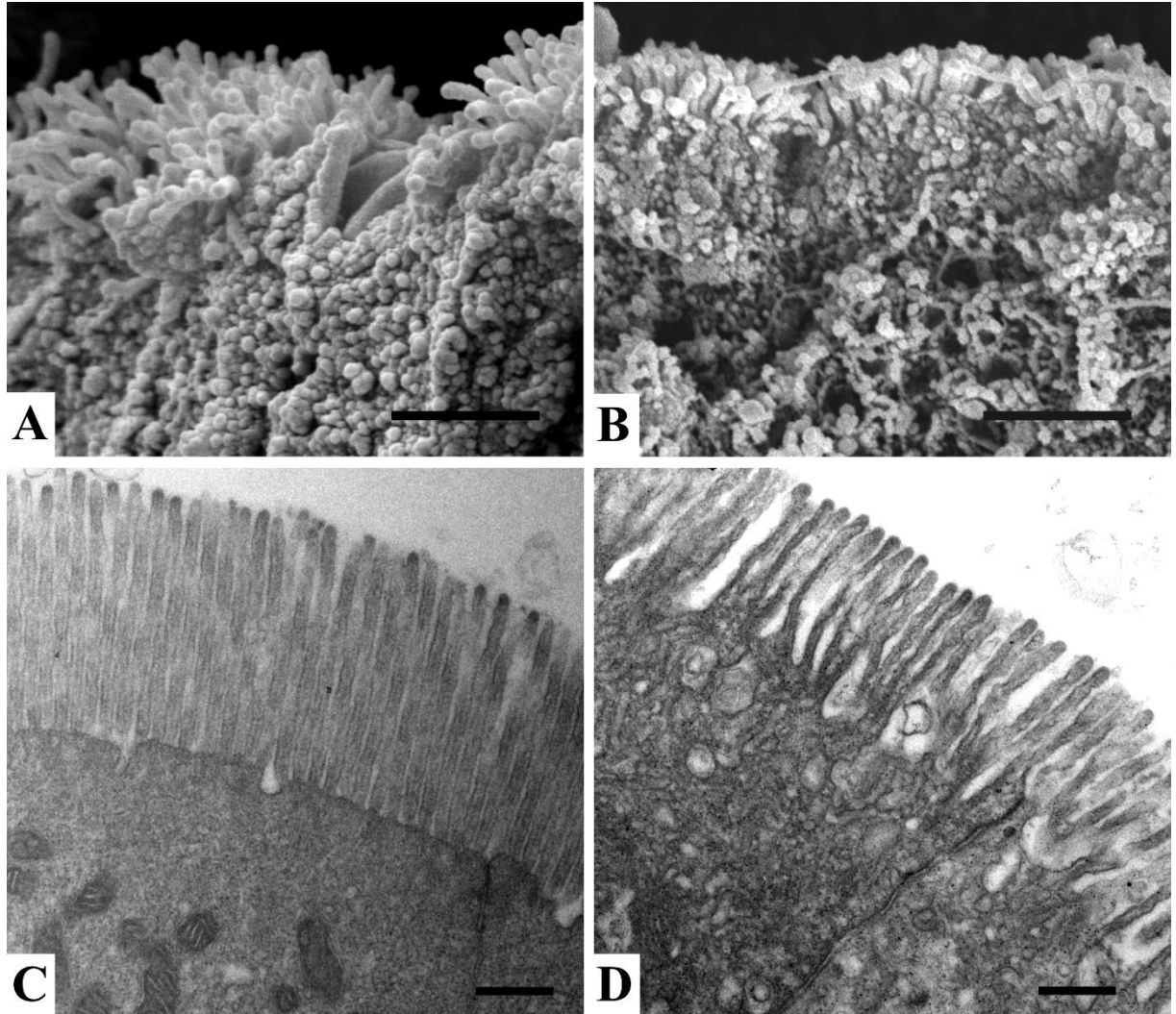


Figure 4. Ultrastructure of the intestine of *Hemisorubim platyrhynchos*. (A and B) Scanning electron micrographs of longitudinal section of the anterior (A) and posterior (B) intestine. Note that microvilli are longer in anterior than in posterior intestine. (C and D) Transmission electron micrographs of anterior (C) and posterior (D) intestine, showing differences in height among these regions. Scale bar: A and B. 2 μ m, C and D. 200nm.

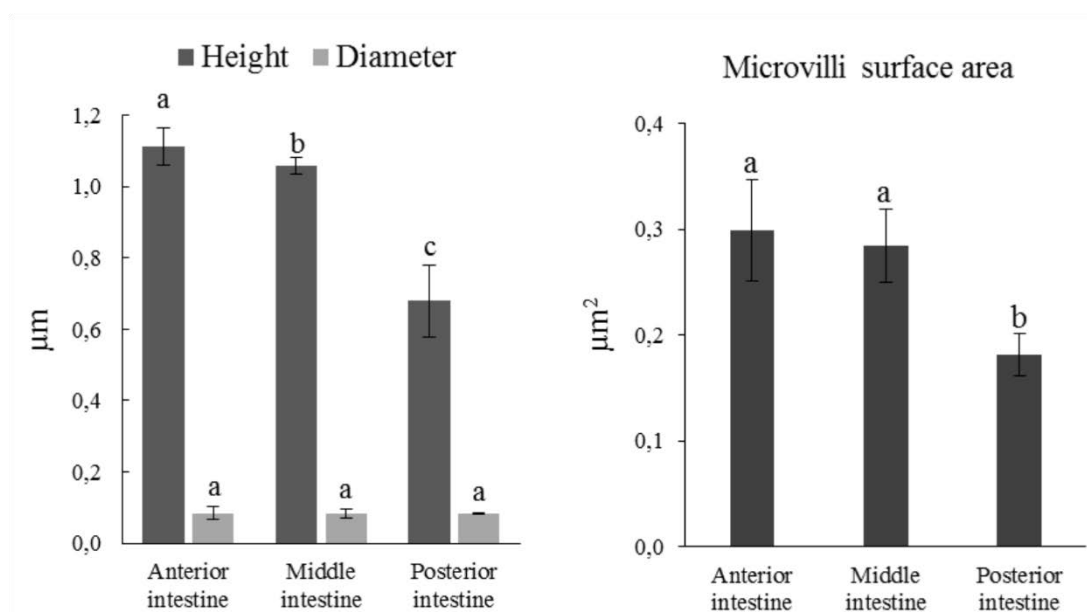


Figure 5. Morphometric data of microvilli height (μm), microvilli diameter (μm) and microvilli surface area (MvSA - μm^2) in the anterior, middle and posterior intestine of *Hemisorubim platyrhynchos*, being made with ANOVA followed by a Tukey's HSD with a family error rate of $P < 0.05$. Values of microvilli height, microvilli diameter and microvilli surface area bars that do not share a common letter are significantly different ($P < 0.05$).

3.3. Capítulo 3 – Artigo enviado para a Revista Micron

Fator de Impacto: 1,988

Cytochemistry of acid and alkaline phosphatase of the digestive tract of the *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae)

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Abstract

This cytochemical study investigated the acid and alkaline phosphatase of the digestive tract of *Hemisorubim platyrhynchos*. Acid phosphatase was detected in the lining epithelium throughout the digestive tract, whereas alkaline phosphatase was concentrated in the intestine. In the esophagus, acid phosphatase reaction occurred in the apical region of the epithelial cells and was associated to epithelial protection and desquamation during renewal epithelial processes. The gastric epithelium showed acid phosphatase in the apical region of the epithelial cells and suggested a role in epithelial protection. In the gastric glands, acid phosphatase was observed in the oxynticopeptic cells in lysosomes which regulated the intracellular lipid stores for use as an energy source and in the vesiculotubular system, associated to secretion processes. Further, acid phosphatase in the intestine was detected in microvilli and lysosomes of the enterocytes and was correlated to absorption and intracellular digestion. However, no difference was reported among the regions of the intestine. On the other hand, alkaline phosphatase reaction revealed a large amount of reaction dots in the anterior intestine with a decrease towards the posterior intestine. This fact suggests that the anterior intestine of *H. platyrhynchos* is the main site of absorption.

Keywords: ultrastructure, brush border, digestive enzyme, Neotropical carnivorous catfish.

3.3.1 Introduction

Studies on the digestion and nutrient absorption processes, especially the characterization and localization of the digestive enzymes, help understand the physiology of the organs of the digestive tract of fish and solve some nutritional problems in fish feeding. According to Kužir et al. (2012), digestion in fish may occur in the gut lumen, at the apical membrane with membrane-bounded enzymes and intracellular in the enterocytes. Consequently, the intestine epithelium presents the brush border forming the junction barrier between the external environment, gut lumen, and the body through which absorbed materials must pass (Crane, 1968). The enzymes involved in food breakdown and absorption lie in the microvilli of the brush border (Kuz'mina and Gelman, 1997). Among them, the predominant enzyme of the fish intestinal brush border is alkaline phosphatase (Silva et al., 2010), which may indicate the intensity of nutrient absorption in the enterocytes.

Further, several authors have also discussed the absorption by pinocytosis and subsequent intracellular digestion by lysosome enzymes (Govoni et al., 1986; Murray et al., 1996). According to Wilson and Castro (2010), the distal intestine of fish may be associated

with the macromolecular protein uptake. Hernandez-Blazquez and Cunha da Silva (1998) concluded that, in *Notothenia neglecta*, the rectum is the intestinal segment where endogenous or exogenous proteins are normally absorbed in great quantity by pinocytosis. In this sense, intracellular digestion is associated to lysosomes and acid phosphatase is a marker enzyme of this organelle (Palmer and Bonner, 2007). Several researchers have used histochemical analyses to describe the enzymatic features of the digestive tract in fish (Tengjaroenkul et al., 2000; Arellano et al., 2001; Kozarić et al., 2004; Mir and Channa, 2010), but cytoenzymological studies by transmission electron microscope are scarce in the literature. The enzymatic ultrastructural analysis provides precise localization data of the enzymes and, thus, enables detailing of the cellular physiology of fish digestive tract.

Hemisorubim platyrhynchos, popularly called “jurupoca” in Brazil, belongs to the Pimelodidae family of the Siluriformes order. It is a migratory species, without parental care, widely distributed throughout Neotropical regions, with reports in the Orinoco, Amazon, Paraguay, Uruguay and Paraná river basins. According to Bressan et al. (2009), *H. platyrhynchos* is a nocturnal carnivorous fish. Similar to other Pimelodidae, *H. platyrhynchos* preys on other fish and is therefore important for the functioning of aquatic ecosystems (Penha and Mateus, 2007). In addition, there has been a reduction in the population size of the species due to habitat destruction in the wake of hydroelectric dams that interrupt the migration flow required for reproduction (Bressan et al., 2009). The species is valuable for aquaculture because of the quality and flavor of its meat and the absence of intramuscular bones. Moreover, the total catch of *H. platyrhynchos* was 50.0 tons in the upper Paraguay River Basin in the state of Mato Grosso do Sul, Brazil, between 1994 and 1999 (Catella, 2001). Although Faccioli et al. (2014) have studied the morphology of the digestive tract of *H. platyrhynchos*, no analysis on the digestive enzymes in this species has been published. Current study analyzed the cytochemistry of the acid and alkaline phosphatase of the digestive tract of *H. platyrhynchos* to better understand the digestion and absorption processes in Neotropical carnivorous catfish.

3.3.2 Materials and Methods

3.3.2.1 Animals

Fifteen adult specimens of *H. platyrhynchos*, with a total body length of 37.4 ± 3.4 cm, were obtained from Pirai Pisciculture in Terenos, MS Brazil. The animals were maintained fasting for one day to empty their digestive tract. They were then anesthetized and euthanized with an overdose of benzocaine, and dissected with a longitudinal incision along the ventral

region. Fragments of esophagus, stomach (cardiac, fundic and pyloric regions) and intestine (anterior, middle and posterior) were fixed in 1% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 1 h, at 4 °C.

3.3.2.2 Detection of acid phosphatase

The fixed fragments were rinsed in 0.1 M sodium cacodylate buffer, pH 7.2, and then incubated at 37 °C for 1 h in 25 mg of cytidine-5'-monophosphate, 12 mL distilled water, 10 mL 0.05M acetate buffer (pH 5.0) and 3 mL 1% lead nitrate (Pino et al., 1981). After incubation, the samples were once again fixed in 2.5% glutaraldehyde, in 0.1 M sodium cacodylate buffer, pH 7.2; they were then post-fixed in 1% osmium tetroxide in the same buffer for 2 h, in the dark. Samples were rinsed again several times in 0.1 M sodium cacodylate buffer and block-stained with an aqueous solution of 2% uranyl acetate for 2 h. Samples were then dehydrated in a graded acetone series and embedded in Araldite resin. Ultra-thin sections (60-80 nm) were mounted on copper grids and analyzed without post-staining. Controls were incubated in medium without substrate. The photodocumentation were performed with a Philips® CM100 transmission electron microscope (Netherlands).

3.3.2.3 Detection of alkaline phosphatase

Fixed fragments were rinsed in 1.4 ml 0.2 M Tris-HCl buffer (pH 8.5), supplemented with 8% sucrose. The samples were then incubated at 37 °C for 1 h in 0.2 M Tris-HCl buffer (pH 8.5), 1 mM β -glycerophosphate, and 2 mM CeCl_3 (Robinson and Karnovsky, 1983). After incubation, the samples were once again fixed in 2.5% glutaraldehyde, in 0.1 M sodium cacodylate buffer, pH 7.2 and then post-fixed in 1% osmium tetroxide, in the same buffer for 2 h, in the dark. Afterwards, the samples were rinsed again several times in 0.1 M sodium cacodylate buffer and block-stained with an aqueous solution of 2% uranyl acetate for 2 h. Subsequently, they were dehydrated in a graded acetone series and embedded in Araldite resin. Ultra-thin sections (60-80 nm) were mounted on copper grids and analyzed without post-staining. Controls were incubated in medium without substrate. The photodocumentation was performed with a Philips® CM100 transmission electron microscope (Netherlands).

3.3.2.4 Ethical note

Current study was approved by the Committee for Ethics in Research of the Institute of Biosciences, Letters and Exact Sciences – UNESP, S. J. Rio Preto SP Brazil, by Protocol n. 075/2013.

3.3.3 Results

Cytoenzymatic reactions revealed that acid phosphatase was located in the lining epithelium throughout the digestive tract of *H. platyrhynchos*, whereas alkaline phosphatase was concentrated in the intestine. The esophagus is a short organ lined by stratified squamous epithelium with three main cellular types: epithelial, goblet and club cells. The epithelial cells lay in all the strata of the epithelium, forming a continuous apical layer of cells. In the apical epithelial cells, the acid phosphatase reaction was detected in the apical cytoplasm forming small electrondense dots near microridges (Fig. 1A and 1B). The nucleus of the epithelial, goblet and club cells also reacts to acid phosphatase, more intensely in the epithelial and goblet cells (Fig. 1C-1E). The cytoplasm of basal epithelial cells and the cytoplasm of goblet and club cells did not react to acid phosphatase.

Gastric mucosa presents a simple columnar epithelium and developed gastric glands in the cardiac and fundic regions. The gastric epithelial cells react to acid phosphatase among the secretory granules, but mainly in contact with the plasma membrane, forming a continuous electrondense reaction (Fig. 2A and 2C). Intense reaction to acid phosphatase was reported in the nucleus of epithelial cells (Fig. 2B). The gastric glands are composed of oxynticopeptic cells that showed cytoplasm with lysosomes and lamellar bodies which were reactive to acid phosphatase (Fig. 2D-2F). Moreover, the apical cytoplasmic region presented a vesiculotubular system that reacted to acid phosphatase in some membranes (Fig. 2G). The nucleus of oxyntic peptic cells also reacted to acid phosphatase (Fig. 2F).

The intestine was divided into three regions: anterior, middle and posterior. Intestine was lined by a simple columnar epithelium with enterocyte and goblet cells. In current cytoenzymological study, the goblet cells did not react to acid or alkaline phosphatase. Although acid phosphatase reaction was detected throughout the intestine in the enterocyte cells, this reaction showed no variation in intensity or in the amount of reaction dots along the intestine (Fig. 3). The basal nucleus of enterocytes showed intense reaction to acid phosphatase (Fig. 3A and 3B). The cytoplasm demonstrated few rounded electrondense vesicles reactive to

this enzyme (Fig. 3C and 3D). Acid phosphatase was also detected in the round enterocyte microvilli (Fig. 3E-3G).

Alkaline phosphatase was detected in enterocyte throughout the intestine but this reaction showed clearly variations in the amount of dots along the intestine. The most conspicuous localization of alkaline phosphatase reaction was detected in the microvilli (Fig. 4A-4F). Large amounts of reaction dots were reported in the anterior intestine, whereas dots decreased in number towards the posterior intestine (Fig. 4A-4F). Moreover, small reaction dots were detected in the cytoplasm of enterocytes. Acid and alkaline phosphatase reactions were not reported in the control groups (Fig. 4G). The cytoenzymological data are summarized in Table 1.

3.3.4 Discussion

In current cytoenzymological study, *H. platyrrhynchus* showed an acid phosphatase reaction in the lining epithelium throughout the digestive tract, whereas alkaline phosphatase was concentrated in the intestine. Acid phosphatase reaction was detected in the cytoplasm of esophageal and gastric epithelial cells, in the cytoplasm of oxyntic peptic cells, and in the microvilli and cytoplasm of enterocytes. Further, acid phosphatase was also registered in the nucleus of the cells of the mucosa layer throughout the digestive tract, mainly in gastric epithelial cells, oxyntic peptic cells and enterocyte cells. Several researchers have reported the nuclear acid phosphatase (Calzada et al., 1998; da Cruz Landim et al., 2002; Anhê et al., 2007; Nóbrega and Quagio-Grassiotto, 2007; Zhu et al., 2008; Guimarães and Quagio-Grassiotto, 2008). According to Nóbrega and Quagio-Grassiotto (2007), *Serrasalmus spilopleura* showed acid phosphatase reaction in the nucleus of Leydig cells, interpreted as a variation of the nuclear functional state. Consequently, acid phosphatase in the nucleus could regulate gene expression by activating or inhibiting some key enzymes related to the gene expression (da Cruz Landim et al., 2002; Guirong et al., 2007).

Acid phosphatase was detected in the apical epithelial cells in the esophagus of *H. platyrrhynchus*. The enzyme was also observed in the esophageal epithelial cells of *Catla catla* (Chakravorty and Sinha, 1982), *Perca fluviatilis* (Hirji, 1983), *Heteropneustes fossilis* and *Cirrhinus reba* (Sastry, 1975), *Solea senegalensis* (Arellano et al., 2001), *Merluccius merluccius* (Kozarić et al., 2004). Although some authors have associated fish esophageal epithelial cells to pre-gastric digestion (Cao and Wang, 2009), acid phosphatase in the esophageal epithelial cells of *H. platyrrhynchus* was located in the apical region, near the

microridges which acted in the esophageal epithelium to facilitate the passage of food, protect the epithelial surface against mechanical injury and anchor the mucins secreted by goblet cells (Sperry and Wassersug, 1976; Humbert et al., 1984; Grau et al., 1992; Abaurrea-Equisoain and Ostos-Garrido, 1996; Soares et al., 1995; Arellano et al., 2001; Faccioli et al., 2014). Nevertheless, esophageal epithelial cells of *H. platyrhynchos* are constantly subject to abrasion, since it is a carnivorous species that ingest large prey. According to Hopwood et al. (1978), the presence of acid phosphatase in human normal esophageal epithelium is associated with the processes of desquamation of surface cells. Thus, the presence of acid phosphatase in esophageal epithelial cells of *H. platyrhynchos* suggests a role in the epithelial desquamation during cellular renewal processes.

Gastric epithelial cells of *H. platyrhynchos* also showed acid phosphatase reaction, particularly among the secretory granules and associated to the apical plasma membrane. In *Merluccius merluccius*, acid phosphatase activity was very strong in the supranuclear region of gastric epithelial cells (Kozarić et al., 2004). Arellano et al. (2001) also reported reaction for this enzyme in the mucosa and lamina propria-submucosa of the stomach of *Solea senegalensis*. Since acid phosphatase in the gastric mucosa has been associated with the epithelial protection against harmful agents (Kent et al., 1966), the reaction of acid phosphatase in the apical region of the gastric epithelial cells of *H. platyrhynchos* emphasizes that the enzyme is also involved in epithelial protection. In the oxyntic peptic cells of gastric glands, the acid phosphatase reaction was detected in lysosomes, lamellar bodies and vesiculotubular system. According to Kozarić et al. (2004), the acid phosphatase of gastric glands of *Merluccius merluccius* is probably connected with the secretion process. Guimarães and Quagio-Grassiotto (2008) suggested that the multilamellar bodies acid phosphatase positives are possibly residual bodies, resulting from the recycling of intracellular components in autophagosomes. The lysosome is the cell's main digestive compartment to which all sorts of macromolecules are delivered for degradation (Lüllmann-Rauch, 2005). In this sense, Singh et al. (2009) registered that nutrient deprivation induces autophagy in mouse hepatocytes, which delivers intracellular proteins and organelles for degradation, but also regulates intracellular lipid stores for use as energy source. In the oxynticopeptic cells of *H. platyrhynchos*, acid phosphatase of the vesiculotubular system may be involved in secretion processes, whereas in lysosomes it probably acts in the degradation of macromolecules for use as an energy source, since these cells require high energy demand.

In current cytoenzymatic study, the intestine of the *H. platyrrhynchus* was divided into anterior, middle and posterior. Acid phosphatase reaction was detected in the lysosomes and microvilli of the enterocytes throughout the intestine. The wide distribution of acid phosphatase in the enterocytes may be correlated to the absorption and transport of metabolites and with the feeding habit of the fish (Mir and Channa, 2010). According to Peters et al. (1990), lysosome acid phosphatase is synthesized as a transmembrane protein that presents a tyrosine containing a endocytosis signal in the cytoplasmic tail which is necessary and sufficient for targeting the lysosomes. Moreover, lysosome acid phosphatase is involved in internalization from the plasma membrane via the clathrin-coated pits (Sosa et al., 1993). Acid phosphatase is also one of the marker enzymes of lysosomes (Palmer and Bonner, 2007). The strong acid phosphatase activity in the intestine of *Schizothorax curvifrons* may be associated to intracellular digestion and absorption of proteins of the ingested diet (Mir and Channa, 2010). Several authors have suggested pinocytotic absorption and intracellular protein digestion mainly in the posterior region of the intestine as an alternative pathway of protein digestion in teleosts (Govoni et al., 1986; Murray et al., 1996; Sarasquete et al., 2001). On the other hand, current cytoenzymatic study revealed no difference in the quantity or intensity of acid phosphatase in lysosomes among the intestinal regions. However, in a morphological study with same species, Faccioli et al. (2014) observed a higher concentration of lysosomes in the posterior intestine. These different results may be due to the one-day fasting that the animals were submitted to for the emptying of the digestive tract. According to Wilson and Castro (2010), the quantitative significance of pinocytotic protein uptake for the intracellular digestion is questionable and most absorption occurs in the fish's anterior intestine. Consequently, current study showed a strong alkaline phosphatases reaction with regard to acid phosphatases in the intestine of *H. platyrrhynchus*, as observed in *Sparus aurata* larvae (Moyano et al., 1996). These authors suggested a greater significance of the intestinal absorption of macromolecules in relation to pinocytic processes.

Alkaline phosphatase reaction was observed in the enterocytes microvilli throughout the intestine of *H. platyrrhynchus*. This enzyme is related to cell membranes, where active transport takes place, and acts in nutrient absorption (Roubaty and Portmann, 1988; Dupuis et al., 1991; Gawlicka et al., 1995; Lallès, 2010; Moraes and Almeida, 2014). Villanueva et al. (1997) concluded that an important function of alkaline phosphatase in the intestine of *Cyprinus carpio* was the dephosphorylation of nutritional compounds, especially when the high amount of the

enzyme in the first intestinal segment was taken into account. Current study revealed a large amount of dots of alkaline phosphatase reaction in the anterior intestine decreasing towards the posterior intestine of *H. platyrrhynchus*. Similar results were observed in *Heteropneustes fossilis* (Sastry, 1975), *Colisa fasciatus* and *Macrognathus aculeatus* (Tyagi et al., 1980), *Oreochromis niloticus* (Smith et al., 2000), hybrid tilapia (Hakim et al., 2006). Results of this cytoenzymological study conclude that the anterior intestine of *H. platyrrhynchus* is the main site of absorption. Further, according to Lallès (2010), intestinal alkaline phosphatase participates in the regulation of surface pH and bicarbonate secretion. Consequently, the strong alkaline phosphatase reaction in the anterior intestine of the studied species suggests an intestinal pH control and contributes towards the protection intestinal mucosa against gastric acid injury, since *H. platyrrhynchus* is a carnivorous species with several gastric glands (Faccioli et al., 2014). In a recent review on intestinal alkaline phosphatase, Lallès (2014) concluded that stimulation of enzyme activity by dietary intervention was a goal for the preservation of gut homeostasis and health by minimizing low-grade inflammation.

In summary, current investigation described the precise location of acid and alkaline phosphatase in the digestive tract of *H. platyrrhynchus*, highlighting the role of acid phosphatase in the renewal of the esophageal epithelium, in the protection of the gastric epithelium and in the degradation of macromolecules in the gastric glands cells for use as an energy source. In turn, alkaline phosphatase was present throughout the intestine, even though an intense reaction was observed in anterior intestine. This fact underscores its importance in maintaining homeostasis and, more particularly, highlights its role in absorption, establishing the anterior intestine as the main absorption site in this carnivorous species.

3.3.5 Acknowledgements

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3.3.6 References

- Abaurrea-Equisoain, M.A., Ostos-Garrido, M.V., 1996. Cell types in the esophageal epithelium of *Anguilla anguilla* (Pisces, Teleostei). Cytochemical and ultrastructural characteristics. *Micron* 27, 419–429.
- Anhê, A.C.B., Lima-Oliveira, A.P.M., Azeredo-Oliveira, M.T.V., 2007. Acid phosphatase activity distribution in salivary glands of triatomines (Heteroptera, Reduviidae, Triatominae). *Genet. Mol. Res.* 6, 197–205.
- Arellano, J.M., Storch, V., Sarasquete, C., 2001. A histological and histochemical study of the oesophagus and oesogaster of the Senegal sole, *Solea senegalensis*. *Eur. J. Histochem.* 45, 279–294.
- Bressan, P.M., Kierulff, M.C.M., Sugieda, A.M., 2009. Fauna ameaçada de extinção no estado de São Paulo: Vertebrados. Fundação Parque Zoológico de São Paulo: Secretaria do Meio Ambiente, São Paulo, 646p.
- Calzada, A., Medina, A., de Canales, M.L.G., 1998. Fine structure of the intestine development in cultured sea bream larvae. *J. Fish Biol.* 53, 340–365.
- Cao, X.J., Wang, W.M., 2009. Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. *Anat. Histol. Embryol.* 38, 254–261.
- Catella, A.C., 2001. A pesca no Pantanal de Mato Grosso do Sul, Brasil: Descrição, nível de exploração e manejo (1994 - 1999). PhD Thesis, 351p. Instituto Nacional de Pesquisas da Amazônia- INPA, Manaus.
- Chakravorty, P., Sinha G.M., 1982. Detection and localization of alkaline and acid phosphatases in the digestive system of the adult *Catla catla* (Hamilton), an Indian freshwater major carp by histochemical methods. *Gegenbaurs Morphol Jahrb.* 128, 799-808.
- Crane, R., 1968. Digestive-absorptive surface of the small bowel mucosa. *Annu. Rev. Med.* 19, 57–68.
- da Cruz Landim, C., Reginato, R.D., de Moraes, R.L.M.S., Cavalcante, V.M., 2002. Cell nucleus activity during post-embryonic development of *Apis mellifera* L. (Hymenoptera: Apidae). Intranuclear acid phosphatase. *Genet. Mol. Res.* 1, 131–138.
- Dupuis, Y., Tardivel, S., Porembska, Z., Fournier, P., 1991. Effect of some phosphatase inhibitors on intestinal calcium transfer. *Int. J. Biochem.* 23, 175–180.
- Faccioli, C.K., Chedid, R.A., Amaral, A.C. do, Franceschini Vicentini, I.B., Vicentini, C.A., 2014. Morphology and histochemistry of the digestive tract in carnivorous freshwater *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae). *Micron* 64, 10–19.
- Gawlicka, A., Teh, S.J., Hung, S.S.O., Hinton, D.E., de la Noüe, J., 1995. Histological and histochemical changes in the digestive tract of white sturgeon larvae during ontogeny. *Fish Physiol. Biochem.* 14, 357–371.
- Govoni, J.J., Boehlert, G.W., Watanabe, Y., 1986. The physiology of digestion in fish larvae. *Environ. Biol. Fish.* 16, 59-77.
- Grau, A., Crespo, S., Sarasquete, M.C., de Canales, M.L.G., 1992. The digestive tract of the amberjack *Seriola dumerili* Risso: a light and scanning electron microscope study. *J. Fish Biol.* 41, 287–303.

- Guimarães, A.C.D., Quagio-Grassiotto, I., 2008. Cytochemical characterization of the endomembranous system during oocyte secondary growth in *Serrasalmus spilopleura* (Teleostei, Characiformes, Characidae). *Acta Zool.* 89, 37–46.
- Guirong, L.I.U., Xianjiang, K., Mingshen, G.U.O., Wenjia, S.U.N., Qi, W., 2007. The study on the localization of acid phosphatase activity during spermiogenesis in chinese mitten-handed crab, *Eriocheir sinensis*. *Bull. Mar. Sci.* 9, 13-19.
- Hakim, Y., Uni, Z., Hulata, G., Harpaz, S., 2006. Relationship between intestinal brush border enzymatic activity and growth rate in tilapias fed diets containing 30% or 48% protein. *Aquaculture* 257, 420–428.
- Hernandez-Blazquez, F.J., Cunha da Silva, J.R.M., 1998. Absorption of macromolecular proteins by the rectal epithelium of the Antarctic fish *Notothenia neglecta*. *Can. J. Zool.* 76, 1247–1253.
- Hirji, K.N., 1983. Observations on the histology and histochemistry of the oesophagus of the perch, *Perca fluviatilis* L. *J. Fish Biol.* 22, 145-152.
- Hopwood, D., Logan, K.R., Milne, G., 1978. The light and electron microscopic distribution of acid phosphatase activity in human normal oesophageal epithelium. *Histochem. J.* 10, 159–170.
- Humbert, W., Kirsch, R., Meister, M.F., 1984. Scanning electron microscopic study of the oesophageal mucous layer in the eel *Anguilla anguilla* L. *J. Fish Biol.* 25, 117–122.
- Kent, T.H., Jervis, H.R., Kuhns, J.S.C., 1966. Enzyme histochemistry of acute staphylococcal enterotoxin gastroenteritis in rhesus monkeys. *Am. J. Pathol.* 48, 667-681.
- Kozarić, Z., Kužir, S., Nejedli, S., Petrinc, Z., Srebočan, E., 2004. Histochemical distribution of digestive enzymes in hake, *Merluccius merluccius* L. 1758. *Vet. Arhiv.* 74, 299-308.
- Kuz'mina, V.V., Gelman, A.G., 1997. Membrane-linked digestion in fish. *Rev. Fish. Sci.* 5, 99-129.
- Kužir, S., Gjurčević, E., Nejedli, S., Baždarić, B., Kozarić, Z., 2012. Morphological and histochemical study of intestine in wild and reared European eel (*Anguilla anguilla* L.). *Fish Physiol. Biochem.* 38, 625–633.
- Lallès, J.P., 2010. Intestinal alkaline phosphatase: Multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. *Nutr. Rev.* 68, 323–332.
- Lallès, J.P., 2014. Intestinal alkaline phosphatase: novel functions and protective effects. *Nutr. Rev.* 72, 82–94.
- Lüllmann-Rauch, R., 2005. History and morphology of the lysosome. In: Saftig P. *Lysosomes*. First Ed. Springer. 1–16.
- Mir, I.H., Channa, A., 2010. Histochemical distribution of lipase and acid phosphatase in the intestinal tract of the snow trout, *Schizothorax curvifrons* Heckel. *J. Biol. Sci.* 10, 643-647
- Moraes, G., Almeida, L.C., 2014. Nutrição e aspectos funcionais da digestão de peixes. In: Baldisserotto, B., Cyrino, J.E.P., Urbinati, E.C. *Biologia e fisiologia de peixes neotropicais de agua doce*. First Ed, Funep, UNESP, 233-252.
- Moyano, F.J., Díaz, M., Alarcón, F.J., Sarasquete, M.C., 1996. Characterization of digestive enzyme activity during larval development of gilthead seabream (*Sparus aurata*). *Fish Physiol. Biochem.* 15, 121–130.

- Murray, H.M., Wright, G.M., Goff, G.P., 1996. A comparative histological and histochemical study of the post-gastric alimentary canal from three species of pleuronectid, the Atlantic halibut, the yellowtail flounder and the winter flounder. *J. Fish Biol.* 48, 187–206.
- Nóbrega, R.H., Quagio-Grassiotto, I., 2007. Morphofunctional changes in Leydig cells throughout the continuous spermatogenesis of the freshwater teleost fish, *Serrasalmus spilopleura* (Characiformes, Characidae): An ultrastructural and enzyme study. *Cell Tissue Res.* 329, 339–349.
- Palmer, T., Bonner, P.L. 2007. *Enzymes: Biochemistry, Biotechnology, Clinical Chemistry*. Second Ed.
- Penha, J.M.F., Mateus, L.A.F. 2007. Sustainable harvest of two large predatory catfish in the Cuiabá river basin, northern Pantanal, Brazil. *Braz. J. Biol.* 67, 81-89.
- Peters, C., Braun, M., Weber, B., Wendland, M., Schmidt, B., Pohlmann, R., Waheed, a, von Figura, K., 1990. Targeting of a lysosomal membrane protein: a tyrosine-containing endocytosis signal in the cytoplasmic tail of lysosomal acid phosphatase is necessary and sufficient for targeting to lysosomes. *Embo J.* 9, 3497–3506.
- Pino, R.M., Pino, L.C., Bankston, P.W., 1981. The relationships between the Golgi apparatus, GERL, and lysosomes of the fetal rat liver Kupffer cells examined by ultrastructural phosphatase cytochemistry. *J. Histochem. Cytochem.* 29, 1061-1064.
- Robinson, J.M., Karnovsky, M.J., 1983. Ultrastructural localization of several phosphatases with cerium. *J. Histochem. Cytochem.* 31, 1197-1208.
- Roubaty, C., Portmann, P., 1988. Relation between intestinal alkaline phosphatase activity and brush border membrane transport of inorganic phosphate, D-glucose, and D-glucose-6-phosphate. *Pflügers Arch.* 412, 482–490.
- Sarasquete, C., Gisbert, E., Ribeiro, L., Vieira, L., Dinis, M.T., 2001. Glyconjugates in epidermal, branchial and digestive mucous cells and gastric glands of gilthead sea bream, *Sparus aurata*, Senegal sole, *Solea senegalensis* and Siberian sturgeon, *Acipenser baeri* development. *Eur. J. Histochem.* 45, 267–278.
- Sastry, V.K., 1975. Alkaline and acid phosphatase in the digestive system of two teleost fishes. *Anat. Anzeiger* 137, 159-65.
- Silva, F.C.P., Nicoli, J.R., Zambonino-Infante, J.L., Le Gall, M.M., Kaushik, S., Gatesoupe, F.J., 2010. Influence of partial substitution of dietary fish meal on the activity of digestive enzymes in the intestinal brush border membrane of gilthead sea bream, *Sparus aurata* and goldfish, *Carassius auratus*. *Aquaculture* 306, 233–237.
- Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A.M., Czaja, M.J., 2009. Autophagy regulates lipid metabolism. *Nature* 458, 1131–1135.
- Smith, B.J., Smith, S.A., Tengjaroenkul, B., Lawrence, T.A., 2000. Gross morphology and topography of the adult intestinal tract of the tilapia fish, *Oreochromis niloticus* L. *Cells. Tissues. Organs* 166, 294–303.
- Soares, T.R.S., Watanabe, I., Lopes, R.A., Semprini, M., Bahls, A.S., Brentegam, L.G., Satake, T., 1995. Light and scanning electron microscopic study of the digestive tract in the catfish *Pseudoplatystoma corruscans* Agassiz, 1829 (Pisces, Pimelodidae). *Braz. J. Morphol. Sci.* 12, 101-108.

- Sosa, M. a, Schmidt, B., von Figura, K., Hille-Rehfeld, a, 1993. In vitro binding of plasma membrane-coated vesicles adaptators to the cytoplasmic domain of lysosomal acid phosphatase. *J. Biol. Chem.* 268, 12537–12543.
- Sperry, D.G., Wassersug, R.J., 1976. A proposed function for microridges on epithelial cells. *Anat. Rec.* 185, 253-257.
- Tengjaroenkul, B., Smith, B.J., Caceci, T., Smith, S., 2000. Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L. *Aquaculture* 182, 317–327.
- Tyagi, M.P., Dalela, R.C., Verma, S.R., 1980. Histochemical mapping of alkaline phosphatase in the digestive system of a few teleost fishes. *Z Mikrosk Anat Forsch* 94, 21-32.
- Villanueva, J., Vanacore, R., Amthauer, R., 1997. Intestinal alkaline phosphatase of the fish *Cyprinus carpio*: Regional distribution and membrane association. *J. Exp. Zool.* 279, 347–355.
- Wilson, J.M., Castro, L.F.C., 2010. Morphological diversity of the gastrointestinal tract in fishes. In: Grosell, M., Farrell, A.P., Brauner, C.J. *The multifunctional gut of fish*. First Ed. Elsevier. 1-55.
- Zhu, J.Y., Ye, G.Y., Hu, C., 2008. Molecular cloning and characterization of acid phosphatase in venom of the endoparasitoid wasp *Pteromalus puparum* (Hymenoptera: Pteromalidae). *Toxicon* 51, 1391–1399.

3.3.7 Figure legends

Table 1. Cytochemical analyses of the digestive tract of *H. platyrhynchos*.

	Esophagus		Stomach	Anterior intestine		Middle intestine		Posterior intestine	
Acid phosphatase	+	++	+	+	++	+	++	+	++
Alkaline phosphatase	-	-	-	+++	-	++	-	+	-
	Epithelial cell	Epithelial cell	Oxynticopeptic cell	Microvilli	Lysosome	Microvilli	Lysosome	Microvilli	Lysosome

Intensity: (-) negative; (+) weak; (++) moderate; (+++) strong.

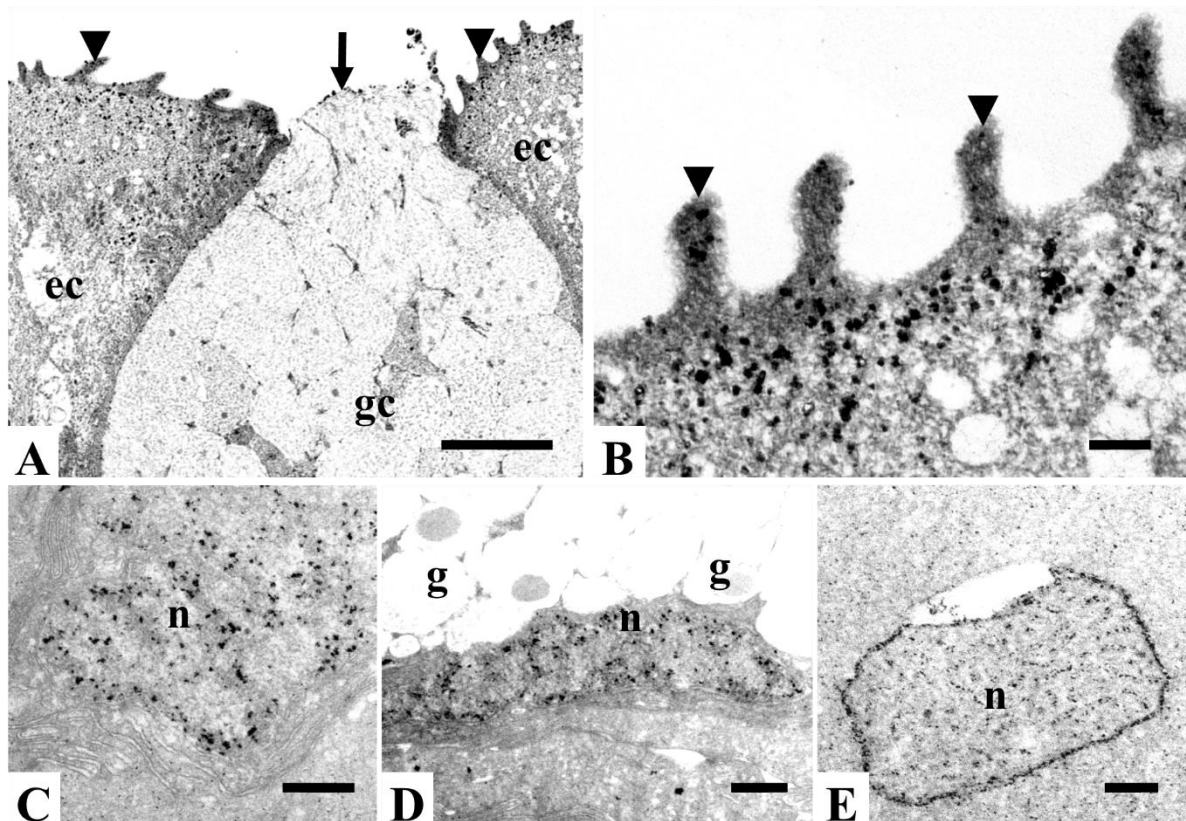


Figure 1. Acid phosphatase reaction in the esophagus of *H. platyrhynchos*. A and B: Esophageal epithelial cell showing small dots of reaction near microridges. C: Nucleus of epithelial cell with small dots of reaction. D: Nucleus of goblet cell, evidencing small electron-dense dots. E: Club cell nucleus, showing small electron-dense dots. Legends: ec-epithelial cell; gc-goblet cell; n-nucleus; g-granules; arrow-opening of goblet cell; arrowhead-microridge. Bars: A. 2µm; B. 200nm; C and D. 500nm; E. 200nm.

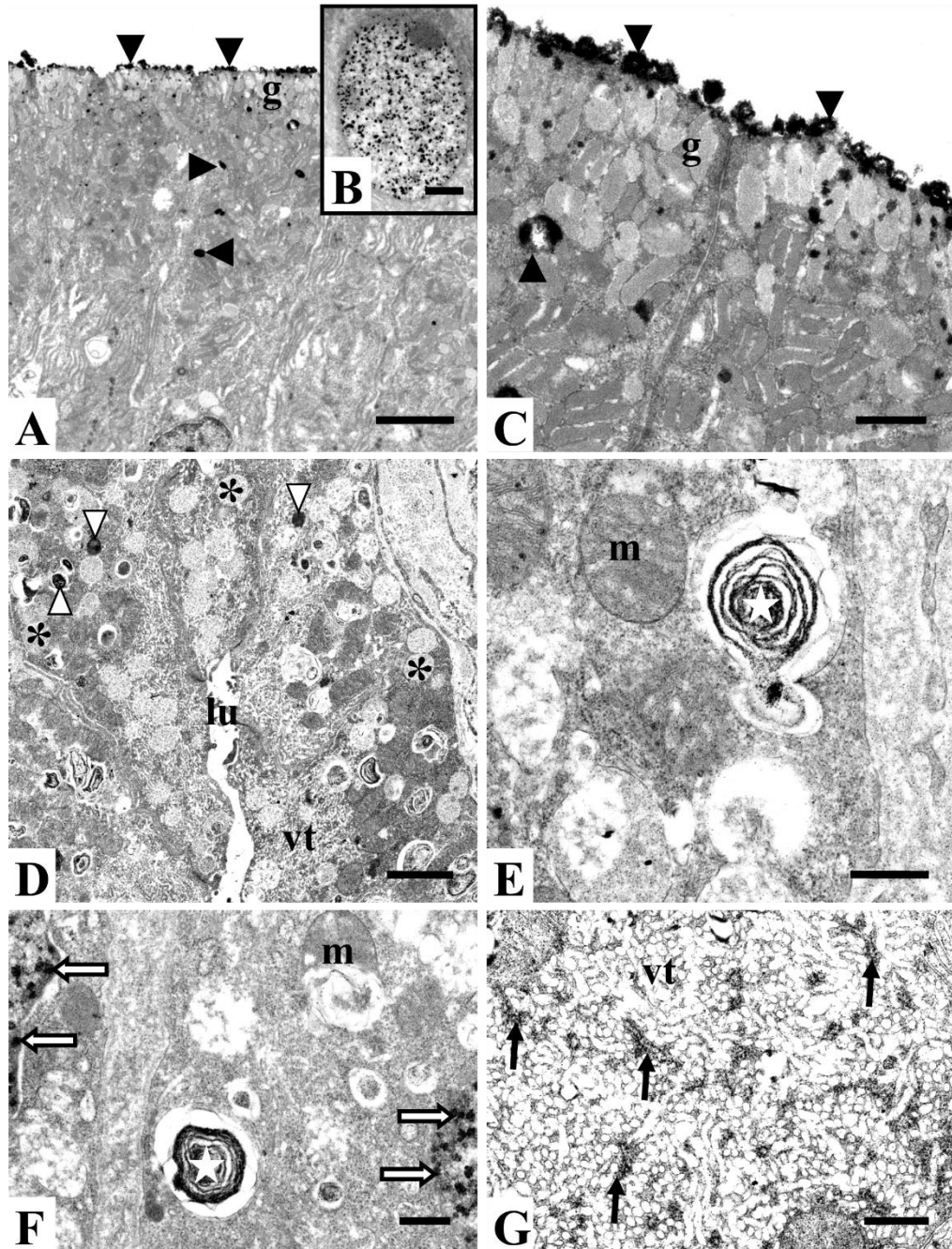


Figure 2. Acid phosphatase reaction in the stomach of *H. platyrhynchos*. A: Gastric epithelial cells showing small electron-dense dots among secretory granules and in the apical surface. B: Nucleus of epithelial cell with many electron-dense dots. C: Apical region of epithelial cells, evidencing electron-dense dots in the apical surface. D, E and F: Oxynticopeptic cells showing cytoplasm with electron-dense lysosomes and lamellar bodies reactive to acid phosphatase. G: Apical region of oxynticopeptic cells, evidencing reaction in some membranes of the vesiculotubular system. Legends: g-granules of epithelial cell; lu-lumen; m-mitochondria; vt-vesiculotubular system; star- lamellar bodies; arrow-reaction in membranes of the vesiculotubular system; white arrow-dots in nucleus of oxynticopeptic cell; arrowhead-reaction dots in epithelial cells; white arrowhead-lysosomes in oxynticopeptic cell; asterisk-zymogene granule. Bars: A. 2 μ m; B. 1 μ m; C. 500nm; D. 2 μ m; E, F and G. 500nm.

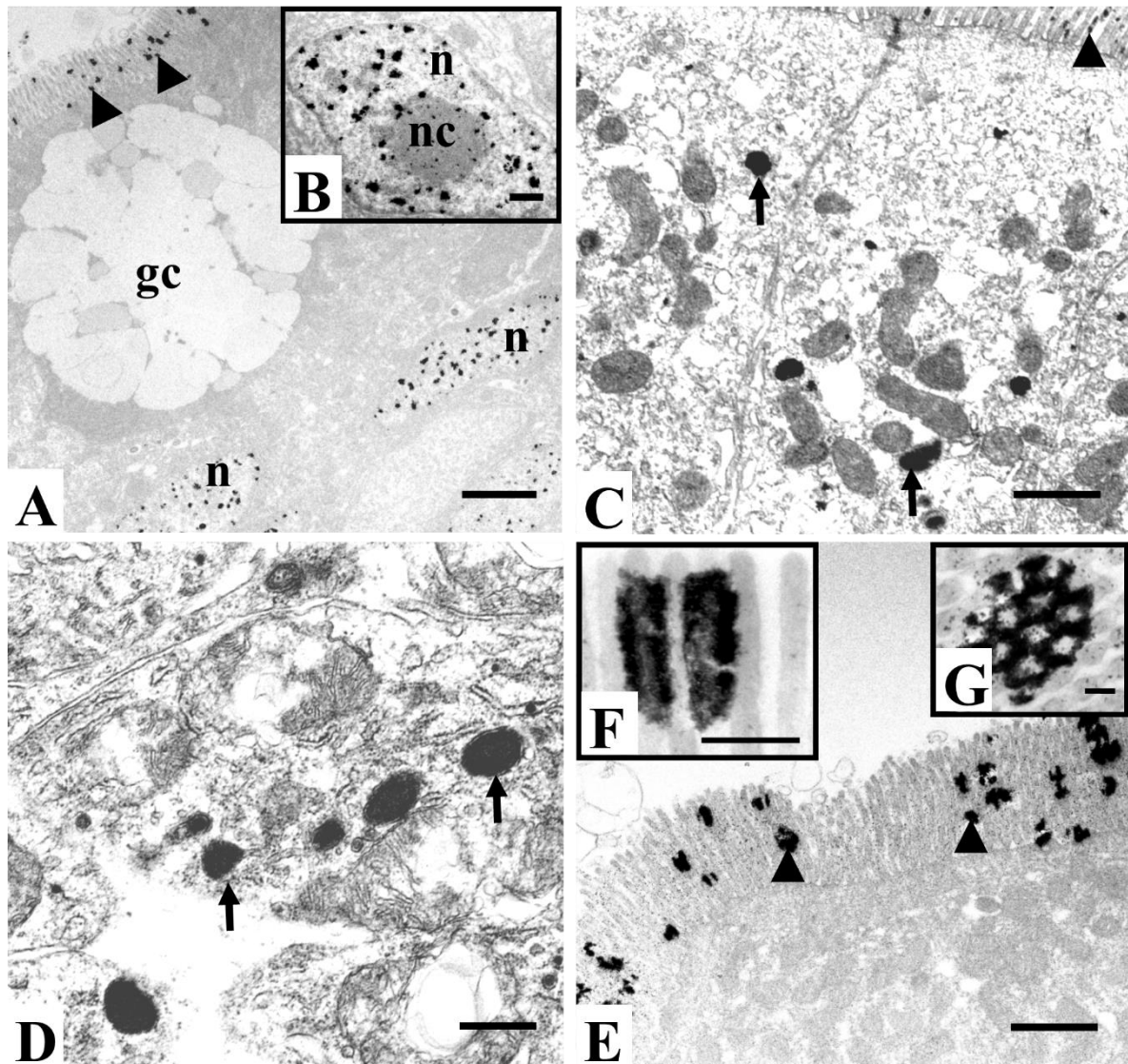


Figure 3. Acid phosphatase reaction in the intestine of the *H. platyrrhynchus*. A: Anterior intestine, showing reaction in the microvilli and nucleus of enterocytes and reaction absence in goblet cells. B: Reaction in the nucleus of enterocytes. C and D: Enterocytes lysosomes, evidencing electron-dense reaction. E, F and G: Electron-dense reaction around enterocytes microvilli. Legends: gc-goblet cell; n-nucleus; nc-nucleolus; arrow-lysosome; arrowhead-reaction around microvilli. Bars: A. 2µm; B. 500nm; C. 1µm; D. 500nm; E. 1µm; F. 250nm; G. 100nm.

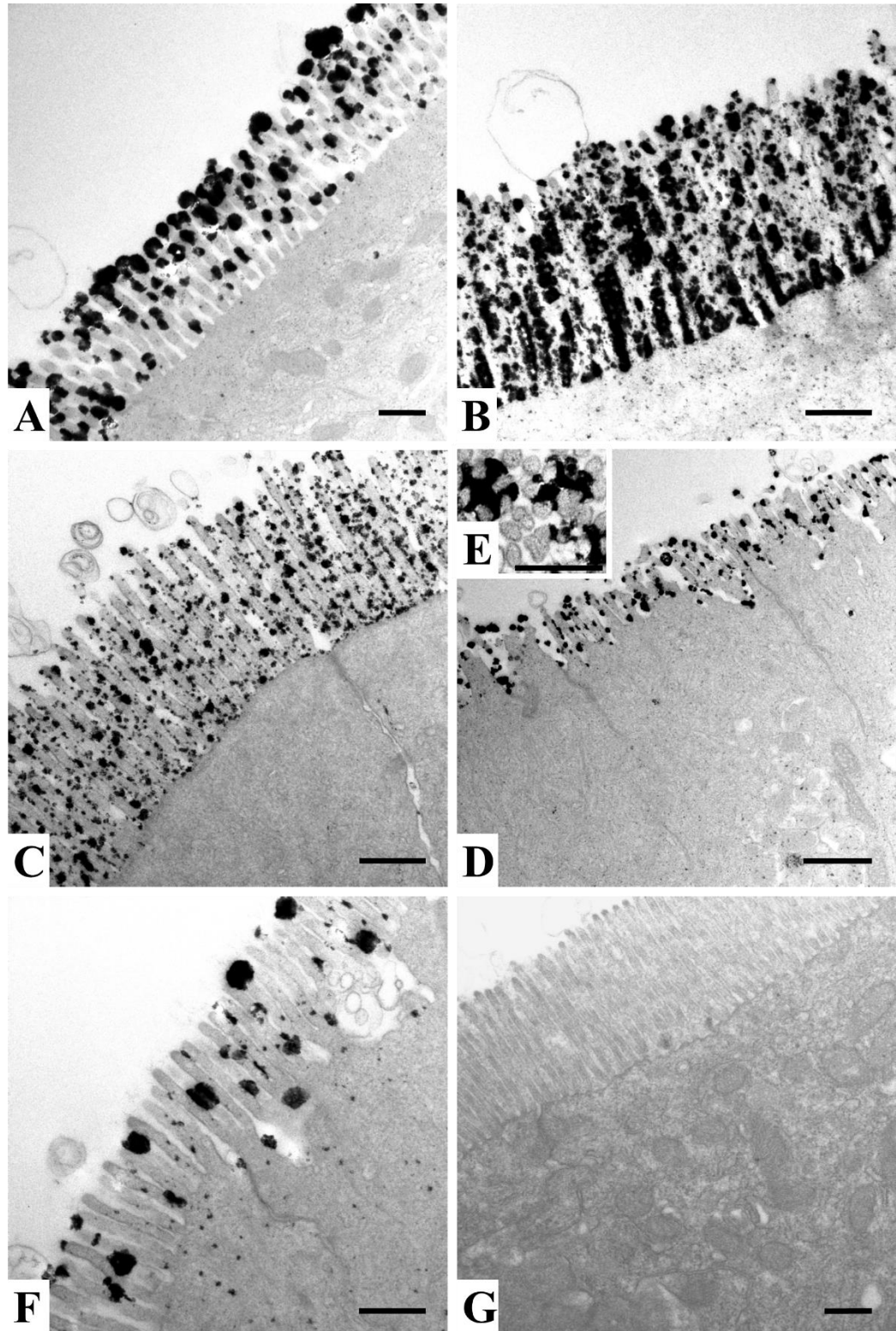


Figure 4. Alkaline phosphatase reaction in the intestine of the *H. platyrrhynchus*. A and B: Anterior intestine, evidencing strong reaction around microvilli. C: Middle intestine, showing a moderate reaction. D, E and F: Posterior intestine, note the weak reaction around microvilli. G: Control group, without reaction. Bars: A, B and C. 500nm; D. 1µm; E. 250nm; F and G. 500nm.

3.4. Capítulo 4 – Artigo a ser enviado para a Revista Acta Histochemica

Fator de Impacto: 1,714

Caracterização citoquímica de organelas membranosas, glicoconjugados ácidos e lipídios na mucosa do tubo digestivo de *Hemisorubim platyrhynchos* (Valenciennes, 1840)

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Resumo

O presente estudo analisou as organelas membranosas, os glicoconjugados ácidos e lipídios na mucosa do tubo digestivo de *H. platyrhynchos*, um bagre carnívoro Neotropical, por meio de microscopia eletrônica de transmissão. As células especializadas na produção de muco, células caliciformes esofágicas e intestinais e células epiteliais gástricas, apresentaram complexo de Golgi mais desenvolvido que os demais tipos celulares. Essas células mostraram grânulos de secreção com reação positiva para glicoconjugados ácidos. A superfície das células epiteliais esofágicas e as microvilosidades dos enterócitos também mostraram reação para glicoconjugados ácidos. Os glicoconjugados ácidos no tubo digestivo protegem a superfície epitelial, promovendo aumento da viscosidade das secreções, o que facilita a passagem do alimento, impede a ligação de parasitas na superfície e facilita a sua expulsão por meio de movimentos peristálticos. Por outro lado, os grânulos de secreção das células caliciformes esofágicas e intestinais apresentaram-se apenas revestidos por membrana enquanto que as células epiteliais gástricas mostraram grânulos com uma marcação intensa para lipídios. As células oxintopépticas das glândulas gástricas apresentaram gotículas lipídicas no citoplasma e também na crista e matriz das mitocôndrias, atuando como reserva de energia para estas células que necessitam de alta demanda energética. Ainda, os enterócitos mostraram retículo endoplasmático liso bem desenvolvido, principalmente na região apical das células, próximo às microvilosidades, estando relacionado com absorção e ressíntese de lipídios.

Palavras-chave: ultraestrutura, estudo citoquímico, tubo digestivo, bagre carnívoro.

3.4.1 Introdução

O tubo digestivo de peixes apresenta grande quantidade de células secretoras de muco no epitélio de revestimento. A composição do muco varia entre as espécies e entre as regiões do tubo digestivo (Tibbetts, 1997; Domeneghini et al., 2002; Díaz et al., 2003; Leknes, 2011) e sua caracterização e localização, bem como o conhecimento sobre morfologia das estruturas envolvidas em sua produção pode auxiliar na compreensão de seu papel em cada órgão. Em peixes, as mucossustâncias digestivas, que são predominantemente compostas de glicoconjugados, estão envolvidas em muitos processos fisiológicos, incluindo a lubrificação epitelial, o aumento da eficiência digestiva, a promoção de absorção de macromoléculas, o tamponamento de fluido intestinal, a proteção contra degradação proteolítica e defesa contra bactérias e outros patógenos (Domeneghini et al., 2005). Diversos autores têm descrito as características das células secretoras de muco em peixes, principalmente por estudos

histoquímicos com microscopia de luz (Tibbetts, 1997; Park e Kim, 2001; Pedini et al., 2001; Domeneghini et al., 2005; Marchetti et al., 2006; Cao e Wang, 2009; Leknes, 2011; Faccioli et al., 2014). No entanto, a análise de glicoconjugados por microscopia electrónica de transmissão é escassa em peixes e pode ajudar na compreensão da fisiologia celular da mucosa do tubo digestivo, especialmente em peixes Neotropicais (Canan et al., 2012).

Por outro lado, pesquisadores têm realizado a caracterização lipídica no tubo digestivo em peixes, com foco principal na absorção intestinal (Sire et al., 1981; Hernandez-Blazquez et al., 2006; Borges et al., 2010). De acordo com Hernandez-Blazquez et al. (2006), o intestino pode ser dividido em três segmentos: anterior, onde lipídios são absorvidos; médio, onde macromoléculas de proteínas são transportadas para dentro do epitélio; e posterior ou reto, que transporta água e íons. Entretanto, Wilson e Castro (2010) destacaram que a significância quantitativa da captação de macromoléculas proteicas seguida por digestão intracelular é questionável e absorção em peixes ocorre principalmente no intestino anterior. Nesse sentido, a análise citoquímica de lipídios é uma ferramenta útil para a compreensão da fisiologia digestiva, auxiliando na caracterização da absorção, bem como das reservas de energia e das secreções celulares no tubo digestivo. Ainda, a análise de estruturas envolvidas na produção de secreções celulares é de difícil visualização, principalmente as características do complexo de Golgi. Por sua vez, Hayat et al. (1993) recomendam a utilização do método do tetróxido de ósmio e iodeto de zinco para melhorar a qualidade da preservação de organelas em nível ultraestrutural.

Hemisorubim platyrhynchos, conhecida popularmente como jurupoca, é uma espécie de porte médio pertencente à família Pimelodidae e à ordem Siluriformes. É um peixe teleósteo reofílico, de hábito noturno e sem cuidado parental. Encontra-se amplamente distribuído na região Neotropical, com ocorrência nas bacias do rio Orinoco, Amazonas, Paraguai e Paraná (Lundberg e Littmann, 2003). Por se tratar de um peixe predador, ocupa níveis altos da cadeia trófica, sendo fundamental para o funcionamento dos ecossistemas aquáticos (Penha e Mateus, 2007). Cabe destacar ainda que as populações de *H. platyrhynchos* sofreram consideráveis reduções devido a destruição do habitat, como resultado da construção de hidrelétricas que interrompem o fluxo de migração necessário para a reprodução (Bressan et al., 2009). Estudos recentes vem sendo conduzidos para melhorar o sistema de criação desta espécie, tanto em relação à ontogenia do sistema digestório em larvas (Faccioli et al., 2016), quanto à morfologia do tubo digestório em animais adultos (Faccioli et al., 2014; Faccioli et al., 2015). Esta espécie

demonstra elevado potencial para cultivo, sendo sua carne muito apreciada devido a qualidade, sabor e ausência de ossos intramusculares – espinhos (Faccioli et al., 2014). Assim, o presente estudo teve como objetivo descrever as organelas membranosas, glicoconjugados ácidos e lipídios na mucosa do tubo digestivo de *H. platyrhynchos* por meio de microscopia eletrônica de transmissão.

3.4.2 Material e Métodos

3.4.1 Animais

Dez espécimes adultos de *H. platyrhynchos*, com um comprimento total de 37.4 ± 3.4 cm, foram obtidos na Piscicultura Pirai (Terenos, Mato Grosso do Sul, Brasil). Os animais foram mantidos em jejum de um dia para o esvaziamento do tubo digestivo. Após este período, os peixes foram anestesiados e eutanasiados com uma overdose de benzocaína e, em seguida, dissecados por meio de uma incisão longitudinal ao longo da região ventral. Fragmentos do tubo digestivo foram fixados em soluções apropriadas para cada estudo citoquímico.

3.4.2 Detecção de organelas membranosas: método do tetróxido de ósmio e iodeto de zinco-ZIO

Para detecção de organelas membranosas, fragmentos do tubo digestivo foram fixados “overnight” em solução de glutaraldeído 2,5% em tampão cacodilato de sódio 0,1 M, pH 7,2. Após a fixação, os fragmentos de tecidos foram lavados em tampão de cacodilato de sódio 0,1 M, pH 7,2 e em seguida em tampão Tris pH 4,5 e incubados em solução de tetróxido de ósmio e iodeto de zinco em tampão Tris pH 4,5, durante 18 horas (Reinecke e Walther, 1978). Após incubação, os fragmentos foram desidratados em séries graduadas de acetona e incluídos em resina araldite. A polimerização da resina foi completada em estufa a 60 °C durante 48 horas. Secções ultrafinas (60-80 nm) foram montadas em grades de cobre, contrastadas de solução de citrato de chumbo 0,2% em hidróxido de sódio 1 N durante 2 minuto e analisadas utilizando um microscópio electrónico de transmissão Philips® CM100 (Holanda).

3.4.3 Detecção de glicoconjugados ácidos: método do vermelho de ruténio

Para a detecção de glicoconjugados ácidos, as amostras do tubo digestivo foram fixadas “overnight” em solução de glutaraldeído 2,5% em tampão de cacodilato de sódio 0,1 M, pH 7,2, com vermelho de ruténio (5 mg/ml), conforme preconizado por Luft (1971). Após a fixação, os fragmentos de tecidos foram lavados durante 10 minutos no mesmo tampão, pós-

fixados com tetróxido de ósmio a 1% em tampão de cacodilato de sódio 0,1 M durante 2 horas no escuro. Em seguida, os fragmentos foram desidratados em séries graduadas de acetona e incluídos em resina araldite. A polimerização da resina foi completada em estufa a 60 °C durante 48 horas. Secções ultrafinas (60-80 nm) foram montadas em grades de cobre e analisadas sem contrastação, utilizando um microscópio electrónico de transmissão Philips® CM100 (Holanda).

3.4.4 Detecção de lipídios: método do ósmio imidazol

Para a detecção de lipídios, as amostras do tubo digestivo foram fixadas “overnight” em solução de glutaraldeído 2,5% em tampão de cacodilato de sódio 0,1 M, pH 7,2. Após a fixação, os fragmentos de tecidos foram lavados em tampão de cacodilato de sódio 0,1 M, pH 7,2 durante 10 minutos e, em seguida, lavado em tampão imidazol 0,1 M, pH 7,5 durante 10 minutos (Angermüller e Fahimi, 1982). Os fragmentos foram pós-fixados em solução de tetróxido de ósmio 2% em tampão imidazol 0,1 M, pH 7,5, durante 30 minutos no escuro à temperatura ambiente. O material foi lavado duas vezes durante 10 minutos em tampão de 0,1 M de imidazol, pH 7,5, desidratado em séries graduadas de acetona e incluídos em resina araldite. A polimerização da resina foi completada em estufa a 60 °C durante 48 horas. Secções ultrafinas (60-80 nm) foram montadas em grades de cobre, contrastadas de solução de citrato de chumbo 0,2% em hidróxido de sódio 1 N durante 1 minuto e analisados utilizando um microscópio electrónico de transmissão Philips® CM100 (Holanda).

3.4.5 Nota ética

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa do Instituto de Biociências, Letras e Ciências Exatas - UNESP, Campus de São José do Rio Preto, SP, Brasil, sob o protocolo nº. 075/2013.

3.4.3 Resultados

O tubo digestivo de *H. platyrhynchos* foi dividido, neste estudo, em esôfago, estômago, com as regiões cárdica e fúndica glandulares e região pilórica aglandular e intestino, dividido em anterior, médio e posterior. Em todas as regiões analisadas, células secretoras de muco foram evidenciadas no epitélio de revestimento. No esôfago e intestino foram encontradas as células caliciformes enquanto que, no estômago, foram observadas apenas as células epiteliais.

A reação citoquímica para glicoconjugados ácidos foi positiva em todos os órgãos, mas a intensidade de reação variou de acordo com a região e com o tipo celular.

No esôfago, três tipos celulares compõem o epitélio de revestimento estratificado: células epiteliais, células caliciformes e células “club”. As células epiteliais aparecem em todos os estratos do epitélio e também formam uma camada apical contínua, que reveste a superfície luminal do esôfago. Essas células apresentaram um núcleo central e irregular com envelope nuclear eletrondenso marcado por ZIO (Fig. 1A e 1B). As organelas membranosas estão localizadas principalmente no citoplasma perinuclear. O complexo de Golgi apresentou-se fortemente eletrondenso, constituído por 3-5 cisternas e numerosas vesículas (Fig. 1A e 1B). As células epiteliais apicais apresentaram microvilli digitiformes e, sobre estas estruturas, glicoconjugados ácidos foram detectados formando uma reação contínua na superfície esofágica (Fig. 1C e 1D).

As células caliciformes mostraram núcleo basal arredondado e complexo de Golgi bem desenvolvido entre o núcleo e grânulos de secreção. As cisternas do Golgi apareceram em número de 5-7, com numerosas vesículas arredondadas nas extremidades (Fig. 2A e 2B). O complexo de Golgi mostrou face cis e trans paralelas. Estas células apresentaram grânulos de secreção com reação positiva para glicoconjugados ácidos com eletrondensidade variada, sendo observada reação em muitos grânulos, mas não em todos (Fig. 2C). Os grânulos apresentaram um revestimento de membrana com marcação para lipídios, revelada pelo método do ósmio imidazol, mas o interior do grânulo permaneceu eletronlucente (Fig. 2D). As células “club” também foram marcadas pelo ZIO, mas poucas organelas membranosas foram identificadas perinuclearmente, principalmente mitocôndrias e complexo de Golgi pouco desenvolvido (Fig. 3A e 3B). Essas células não apresentaram reação para glicoconjugados ácidos.

A mucosa gástrica apresentou epitélio de revestimento composto por células colunares, com núcleo no terço basal da célula. O citoplasma apresentou complexo de Golgi bem desenvolvido com 5-7 cisternas e vesículas esféricas pequenas (Fig. 4A-4C). As faces cis e trans mostraram-se curvadas nas extremidades, formando uma superfície convexa e côncava, respectivamente. No citoplasma apical, numerosos grânulos de secreção foram observados, mas nem todos foram reativos para glicoconjugados ácidos (Fig. 4D). Por outro lado, todos esses grânulos mostraram-se fortemente eletrondensos para marcação de lipídios (Fig. 4E). As glândulas gástricas tubulares apresentaram-se compostas por células oxintopépticas, com núcleo basal arredondado. O citoplasma apresentou duas regiões distintas, uma região

perinuclear repleta de mitocôndrias marcadas pelo ZIO e uma região apical com sistema vesiculotubular (Fig. 5A-5C). O sistema vesiculotubular também apresentou membranas marcadas pelo ZIO, sendo entremeadas por grânulos de zimogênio esféricos (Fig. 5C). Ainda, o citoplasma apresentou grânulos eletrondensos reativos para glicoconjugados ácidos (Fig. 5D) e gotículas lipídicas marcadas pelo método do ósmio imidazol (Fig. 5E). A marcação lipídica também foi observada nas cristas mitocondriais e em pequenos pontos na matriz mitocondrial (Fig. 5E).

O intestino mostrou-se revestido por epitélio simples colunar composto principalmente por enterócitos e células caliciformes. Os enterócitos apresentaram núcleo basal e citoplasma com numerosas mitocôndrias e complexo de Golgi bem desenvolvido com 4-7 cisternas (Fig. 6A-6C). O citoplasma apical, próximo às microvilosidades, mostrou retículo endoplasmático liso bem desenvolvido, composto por estruturas tubulares fortemente eletrondensas ao ZIO (Fig. 6A e 6C). Ao redor das microvilosidades, foi evidenciada reação positiva para glicoconjugados ácidos ao longo de todo intestino. Essa reação foi forte no intestino posterior enquanto que no intestino anterior e médio foi moderada (Fig. 6D-6F). Ainda, o citoplasma dos enterócitos mostrou algumas estruturas esféricas eletrondensas com marcação para lipídios (Fig. 6G e 6H). O tamanho e número destas estruturas não variou ao longo das regiões intestinais.

As células caliciformes intestinais apresentaram núcleo basal e citoplasma repleto de grânulos de secreção. O citoplasma perinuclear exibiu complexo de Golgi bem desenvolvido, entre o núcleo e grânulos de secreção composto de 5-8 cisternas (Fig. 7A e 7B). O complexo de Golgi foi observado com a face cis voltada para o núcleo, com formato convexo e face trans côncava voltada para os grânulos. Os grânulos mostraram-se positivos para reação de glicoconjugados ácidos (Fig. 7C). Embora o número de células caliciformes seja maior no intestino posterior, a intensidade desta reação não variou entre as regiões intestinais. Assim como no esôfago, os grânulos das células caliciformes intestinais exibiram um revestimento de membrana com marcação para lipídios, revelada pelo método do ósmio imidazol, mas o interior do grânulo permaneceu eletronlucente (Fig. 7D).

3.4.4 Discussão

O presente trabalho descreveu as características das organelas membranosas, a presença de glicoconjugados ácidos e lipídios ao longo do tubo digestivo de *H. platyrhynchos*. Embora o estudo de Faccioli et al. (2014) tenha descrito as características morfológicas gerais do tubo

digestivo nesta espécie, pouca atenção foi dada ao complexo de Golgi, devido à dificuldade de observação por técnicas convencionais de microscopia. Nesse sentido, o método do tetróxido de ósmio e iodeto de zinco mostrou-se uma ferramenta bastante útil para a visualização de organelas membranosas, permitindo a descrição do complexo de Golgi. As células especializadas na produção de muco, células caliciformes esofágicas e intestinais e células epiteliais gástricas, apresentaram complexo de Golgi mais desenvolvido que os demais tipos celulares, contendo entre 5-8 cisternas. O complexo de Golgi é um dos principais sítios de síntese de carboidratos, bem como uma estação de classificação e de destinação para produtos do retículo endoplasmático (Alberts et al., 2010). Uma análise quantitativa do tamanho da partícula secretada mostrou que esta é influenciada pelo tamanho e topologia do complexo de Golgi, com correspondente influência sobre a função secretora, mostrando que o Golgi não só determina modificações nas proteínas, mas também regula a sua montagem em objetos multiméricos antes da secreção (Ferraro et al., 2014).

Em relação à citoquímica para detecção de glicoconjugados ácidos, no esôfago foi observada reação nas células caliciformes e na superfície das células epiteliais. A função primária do esôfago é transportar os alimentos da bucofaringe para o estômago. Assim, as células epiteliais apicais apresentam microvilli digitiformes que protegem a superfície epitelial contra danos mecânicos e ancoram o muco secretado pelas células caliciformes, formando uma superfície epitelial lubrificada para a passagem de alimentos e para proteção contra patógenos (Sperry e Wassersug, 1976; Humbert et al., 1984; Grau et al., 1992; Abaurrea-Equisoain e Ostos-Garrido, 1996). Em adição, as microvilli aumentam a área da superfície de contato entre a membrana plasmática e as mucossustâncias. De acordo com Cone et al. (2009), o muco adere a superfície das células epiteliais por mecanismos ainda pouco conhecidos que envolvem adesão e numerosas interações químicas entre as mucossustâncias secretadas e as mucossustâncias da superfície das células que formam o glicocálice.

As células caliciformes esofágicas de *H. platyrhynchos* apresentaram com grânulos de secreção com um revestimento de membrana, marcado pelo método do ósmio imidazol enquanto que o interior do grânulo permaneceu eletrólucido. Estes grânulos foram reativos para glicoconjugados ácidos com eletrondensidade variada. Os glicoconjugados ácidos estão associados com o aumento da viscosidade das secreções (Tibbetts, 1997; Díaz et al., 2008), com a lubrificação epitelial para a prevenção de danos mecânicos e proteção contra patógenos (Fletcher e Grant, 1969; Humbert et al., 1984; Abaurrea-Equisoain e Ostos-Garrido, 1996).

Cabe destacar que *H. platyrrhynchus* é uma espécie carnívora que ingere presas inteiras e, portanto, apresenta uma grande quantidade de glicoconjugados para proteger e lubrificar o epitélio esofágico. Por outro lado, considerando que peixes não apresentam glândulas salivares, as mucossustâncias produzidas pelas células caliciformes no esôfago podem proteger o epitélio ao longo de todo tubo digestivo (Scocco et al., 1998).

Na mucosa gástrica de *H. platyrrhynchus*, glicoconjugados ácidos foram detectados no epitélio e nas glândulas. As células epiteliais gástricas mostraram reação para glicoconjugados ácidos, em alguns grânulos de secreção, principalmente perto da membrana plasmática apical. Diversos pesquisadores descreveram as mucossustâncias apicais nas células epiteliais gástricas, principalmente as mucinas neutras (Ferraris et al., 1987; Pedini et al., 2005; Carrassón et al., 2006; Cao e Wang, 2009). Por sua vez, Spicer e Schulte (1992) encontraram mucossustâncias ácidas nas células epiteliais gástricas e associaram com a capacidade para formar um complexo com pepsina e, assim, estabilizar ou tamponar a enzima devido à atividade antipéptica. Em *H. platyrrhynchus*, Faccioli et al. (2014) observaram nas regiões cárdica e fúndica glândulas gástricas desenvolvidas e, assim, os glicoconjugados ácidos são fundamentais para a proteção epitelial contra suco gástrico. De acordo Bansil e Turner (2006), a camada de muco na superfície luminal do estômago forma um gel carregado negativamente que impede a retrodifusão de íons de hidrogênio, confinando o ácido no lúmen do tubo digestivo. Além disso, os grânulos de secreção das células epiteliais gástricas de *H. platyrrhynchus* mostraram marcação eletrondensas para lipídios com o método do ósmio imidazol. De acordo com Słomiany et al. (1985), o muco intracelular da mucosa gástrica de mamíferos, cães e ratos, apresentou-se composto por lipídios, carboidratos e proteínas. Murty et al. (1984) evidenciaram que o muco gástrico de cães contém 26,5% de lipídios associados. Ainda, a camada de muco gástrico não apresenta uma característica única, mas é uma mistura heterogênea de proteínas, glicoproteínas e lipídios sob a forma de um gel embebido com água e eletrólitos (Słomiany e Słomiany, 2004). As células epiteliais gástricas estão ativamente envolvidas na síntese e armazenamento de fosfolipídios de superfície, que são componentes essenciais do revestimento hidrofóbico de proteção do estômago (Kao e Lichtenberger, 1987).

A reação para glicoconjugados ácidos também foi evidenciada nas glândulas gástricas de *H. platyrrhynchus*, atuando provavelmente na proteção da superfície luminal. Além disso, as células oxintopépticas das glândulas contêm gotículas lipídicas no citoplasma e também na crista e matriz mitocondrial. Em *Sparus aurata*, as células das glândulas gástricas apresentaram

algumas gotículas lipídicas (Elbal et al., 2004). No citoplasma basal das células glandulares de *Acipenser naccarii*, gotículas lipídicas foram relativamente abundantes (Camacho et al., 2011). De acordo com Geuze (1971), as gotículas lipídicas atuam como locais de armazenamento de energia e tornam-se operacionais após oxidação mitocondrial, justificando sua presença nas células oxintopépticas de *H. platyrhynchos*, uma vez que estas células requerem elevada demanda de energia.

A mucosa intestinal apresentou epitélio de revestimento constituído principalmente de enterócitos e células caliciformes. No citoplasma dos enterócitos, foi evidenciada a presença estruturas tubulares descontínuas, caracterizando retículo endoplasmático liso, principalmente na região apical das células, próximo às microvilosidades. Gilloteaux e Naud (1979) sugeriram que a técnica do ZIO marca principalmente locais nas membranas contendo íons cálcio, o que reflete esta marcação observada no retículo endoplasmático liso. De acordo com Dunn (1967), a maioria dos enterócitos de ratos possui um vasto retículo endoplasmático liso apical, intimamente relacionado com a absorção dos alimentos. Assim como em mamíferos, em peixes a ressíntese de triglicerídeos a partir de ácidos graxos e monoglicerídeos absorvidos, ocorre em nível do retículo endoplasmático liso (Noaillac-Depeyre e Gas, 1974). Por sua vez, o citoplasma dos enterócitos na espécie estudada apresentou poucas gotículas lipídicas esféricas eletrondensas marcadas pelo método do ósmio imidazol. O tamanho e número destas estruturas não variou ao longo regiões intestinais. Em peixes, a absorção de lipídios ocorre predominantemente nas regiões proximais do intestino (Tocher, 2003; Hernandez-Blazquez et al., 2006; Wilson e Castro, 2010). No entanto, lipídios podem ser absorvidos ao longo de todo o comprimento do intestino, porém diminuindo em quantidade em direção posterior (Tocher, 2003), o que também depende da classe de lipídios, do comprimento da cadeia e do grau de saturação (Bakke et al., 2010).

No epitélio intestinal de *H. platyrhynchos*, glicoconjugados ácidos foram observados ao redor das microvilosidades dos enterócitos, apresentando uma reação mais intensa no intestino posterior. Provavelmente este fato tenha ocorrido devido ao maior número de células caliciformes na região posterior (Faccioli et al., 2014). Sarasquete et al. (2001) observaram reação fraca de mucossubstâncias ácidas na borda em escova intestinal de *Acipenser baeri* e *Sparus aurata* e uma forte reação em *Solea senegalensis*. Esta reação também foi observada em *Pleuronectes ferruginea* (Bagloli et al., 1997) e *Sander lucioperca* (Ostaszewska, 2005). Em *H. platyrhynchos*, os grânulos de secreção das células caliciformes também foram reativos aos

glicoconjugados ácidos, mas a intensidade foi forte em todas as regiões intestinais. Como no esôfago, estes grânulos apresentaram um revestimento de membrana. As mucossubstâncias ácidas têm sido relacionadas com aumento da viscosidade das secreções (Tibbetts, 1997; Diaz et al., 2008), com a proteção do epitélio intestinal contra a ação das glicosidases, aumentando a resistência do muco a degradação bacteriana (Rhodes et al., 1985). Além disso, diversos autores relataram um aumento de glicoconjugados ácidos em peixes com parasitas (Bosi et al., 2005; Dezfuli et al., 2010). Neste sentido, alguns estudos têm descrito a presença de diversos parasitas em *H. platyrrhynchos* (Pavanelli et al., 1997; Guidelli et al., 2002; Guidelli et al., 2003; Chambrier et al., 2006; Vieira et al., 2010; Pádua et al., 2013). De acordo com Bosi et al. (2005), o muco secretado na superfície do intestino forma uma barreira física para prevenção de uma possível ligação de parasitas e facilita a sua expulsão por movimentos peristálticos.

Diante do exposto, foi possível verificar que o tubo digestivo de *H. platyrrhynchos* apresentou glicoconjugados ácidos em todos os órgãos analisados, promovendo a lubrificação da superfície epitelial para facilitar a passagem do alimento, a proteção contra autodigestão e contra patógenos. Por outro lado, as secreções epiteliais gástricas também apresentaram-se marcadas intensamente para lipídios, o que auxilia na proteção epitelial. As células oxintopépticas das glândulas gástricas apresentaram gotículas lipídicas no citoplasma e também na crista e matriz das mitocôndrias, atuando como reserva de energia para estas células que necessitam de alta demanda energética. Ainda, os enterócitos mostraram retículo endoplasmático liso bem desenvolvido, principalmente na região apical das células, estando relacionado com absorção e ressíntese de lipídios.

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3.4.6 Referências Bibliográficas

- Abaurrea-Equisoain, M.A., Ostos-Garrido, M.V. Cell Types in the Esophageal Epithelium of *Anguilla anguilla* (Pisces, Teleostei). Cytochemical and Ultrastructural Characteristics. *Micron*, v.27, p.419-429, 1996.
- Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Biologia molecular da célula*. Quinta ed, Artmed, São Paulo, 1396p, 2010.
- Angermüller, S.; Fahimi, H.D. Imidazole-buffered osmium tetroxide: an excellent stain for visualization of lipids in transmission electron microscopy. *Histochemical Journal*, v.14, p.823-835, 1982.
- Baglolle, C.J.; Murray, H.M.; Goff, G.P.; Wright, G.M. Ontogeny of the digestive tract during larval development of yellowtail flounder: a light microscopic and mucous histochemical study. *Journal of Fish Biology*, v.51, p.120–134, 1997.
- Bakke, A.M.; Glover, C.; Krogdahl, S. Feeding, digestion and absorption of nutrients. In: Grosell, M., Farrell, A.P., Brauner, C.J. (Eds.), *Fish Physiology: The Multifunctional Gut of Fish*. Academic Press, San Diego, p.57-110, 2010.
- Bansil, R.; Turner, B.S. Mucin structure, aggregation, physiological functions and biomedical applications. *Current Opinion in Colloid & Interface Science*, v.11, p.164–170, 2006.
- Borges, J.C.S., Pressionotti, L.N., Vicente Gomes, V., Cunha Da Silva, J.R.M. Lipidic and proteic absorption in digestive tract of tropical fat snook (*Centropomus parallelus*, POEY 1860). *Journal of Experimental Marine Biology and Ecology*, v.386, p.39-44, 2010.
- Bosi, G.; Arrighi, S.; Di Giancamillo, A.; Domeneghini, C. Histochemistry of glycoconjugates in mucous cells of *Salmo trutta* uninfected and naturally parasitized with intestinal helminths. *Diseases of Aquatic Organisms*, v.64, p.45–51, 2005.
- Bressan, P.M.; Kierulff, M.C.M.; Sugieda, A.M. Fauna ameaçada de extinção no estado de São Paulo: Vertebrados. São Paulo: Fundação Parque Zoológico de São Paulo: Secretaria do Meio Ambiente, 646p, 2009.
- Camacho, S.; Carmona, R.; Llorente, J.I.; Sanz, A.; García-Gallego, M.; Domezain, A.; Dominguez, N.; Ostos-Garrido, M.V. Stomach development in the sturgeon *Acipenser naccarii*: histoenzymatic and ultrastructural analysis. *Journal of Applied Ichthyology*, v.27, p.693–700, 2011.
- Canan B, Nascimento WS, Silva NB, Chellappa S. Morphohistology of the Digestive Tract of the Damsel Fish *Stegastes fuscus* (Osteichthyes: Pomacentridae). *Science World Journal*, v.2012, p.1-9, 2012.
- Cao, X.J.; Wang, W.M. Histology and mucin histochemistry of the digestive tract of yellow catfish, *Pelteobagrus fulvidraco*. *Anatomia Histologia Embryologia*, v.38, p.254-261, 2009.
- Carrassón, M. Grau, A.; Dopazo, L.R.; Crespo, S. A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). *Histology and Histopathology*, v.21, p.579-593, 2006.
- Chambrier, A.; Scholz, T.; Kuchta, R.; Posel, P.; Mortenthaler, M.; Guardia, C.C. Tapeworms (Cestoda: Proteocephalidea) of fishes from the Amazon river in Peru. *Comparative Parasitology*, v.73, p.111–120, 2006.

- Cone, R.A. Barrier properties of mucus. *Advanced Drug Delivery Reviews*, v.61 p.75–85, 2009.
- Dezfuli, B.S.; Pironi, F.; Campisi, M.; Shinn, A.P.; Giari, L. The response of intestinal mucous cells to the presence of enteric helminths: their distribution, histochemistry and fine structure. *Journal of Fish Diseases*, v.33, p.481–488, 2010.
- Díaz, A.O.; García, A.M.; Devinenti, C.V.; Goldemberg, A.L. Morphological and histochemical characterization of the mucosa of the digestive tract in *Engraulis anchoita* (Hubbs and Marini, 1935). *Anatomia Histologia Embryologia*, v.32, p.341–346, 2003.
- Díaz, A.O.; García, A.M.; Goldemberg, A.L. Glycoconjugates in the mucosa of the digestive tract of *Cynoscion guatucupa*: A histochemical study. *Acta Histochemica*, v.110, p.76–85, 2008.
- Domeneghini, C.; Arrighi, S.; Radaelli, G.; Bosi, G.; Veggetti, A. Histochemical analysis of glycoconjugates secretion in the alimentary canal of *Anguilla anguilla* L. *Acta Histochemica*, v.106, p.477–487, 2005.
- Domeneghini, C.; Radaelli, G.; Bosi, G.; Arrighi, S.; Di Giancamillo, A.; Pazzaglia, M.; Mascarello, F. Morphological and histochemical differences in the structure of the alimentary canal in feeding and runt (feed deprived) cultured white sturgeons (*Acipenser transmontanus*). *Journal of Applied Ichthyology*, v.18, p.341–346, 2002.
- Dunn, J.S. The fine structure of the absorptive epithelial cells of the developing small intestine of the rat. *Journal of Anatomy*, v.101, p.57–68, 1967.
- Elbal, M.T.; García-Hernández, M.P.; Lozano, M.T.; Agulleiro, B. Development of the digestive tract of gilthead sea bream (*Sparus aurata* L.). Light and electron microscopic studies. *Aquaculture*, v.234, p.215–238, 2004.
- Faccioli, C.K.; Chedid, R.A.; Amaral, A.C.; Franceschini-Vicentini, I.B.; Vicentini, C.A. Morphology and histochemistry of the digestive tract in carnivorous freshwater *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae). *Micron*, v.64, p.10–19, 2014.
- Faccioli, C.K.; Chedid, R.A.; Mori, R.H.; Amaral, A.C.; Belmont, R.A.F.; Franceschini-Vicentini, I.B.; Vicentini, C.A. Organogenesis of the digestive system in Neotropical carnivorous freshwater catfish *Hemisorubim platyrhynchos* (Siluriformes: Pimelodidae). *Aquaculture*, v.451, p.205–212, 2016.
- Faccioli, C.K.; Chedid, R.A.; Mori, R.H.; Amaral, A.C.; Franceschini-Vicentini, I.B.; Vicentini, C.A. Ultrastructure of the digestive tract in neotropical carnivorous catfish *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Siluriformes, Pimelodidae). *Scanning*, p.1–8, 2015.
- Ferraris, R.P.; Tan, J.D.; de la Cruz, M.C. Development of the digestive tract of milkfish, *Channos chanos*. *Aquaculture*, v.61, p.241–257, 1987.
- Ferraro, F.; et al. A two-tier Golgi-based control of organelle size underpins the functional plasticity of endothelial cells. *Developmental Cell*, v.29, p.292–304, 2014.
- Fletcher, T.C., Grant, P.T. Immunoglobulins in the serum and mucus of the plaice (*Pleuronectes platessa*). *Biochemical Journal*, v.115, p.65, 1969.

- Geuze, J.J. Light and electron microscope observations on the gastric mucosa of the frog (*Rana esculenta*) I. Normal structure. Zeitschrift für Zellforschung und mikroskopische Anatomie, v.117, p.87-102, 1971.
- Gilloteaux, J.; Naud, J. The zinc iodide-osmium tetroxide staining-fixative of maillet. Nature of the precipitate studied by X-ray microanalysis and detection of Ca²⁺ affinity subcellular sites in a tonic smooth muscle. Histochemistry, v.63, p.227-243, 1979.
- Grau, A.; Crespo, S.; Sarasquete, M.C.; Gonzales De Canales, M.L. The digestive tract of the amberjack *Seriola dumerili* Risso: a light and scanning electron microscope study. Journal of Fish Biology, v.41, p.287-303, 1992.
- Guidelli, G.M.; Isaac, A.; Pavanelli, G.C. *Sanguinicola platyrhynchi* n. sp. (Digenea: Sanguinicolidae) parasite of visceral cavity of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Pisces: Pimelodidae) from the floodplain of the upper Paraná river, Brazil. Brazilian Journal of Biology, v.62, p.801-806, 2002.
- Guidelli, G.M.; Isaac, A.; Takemoto, R.M.; Pavanelli, G.C. Endoparasite infracommunities of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Pisces: Pimelodidae) of the baía river, upper Paraná river floodplain, Brazil: specific composition and ecological aspects. Brazilian Journal of Biology, v.63, p.261-268, 2003.
- Hayat, M.A. Stains and Cytochemical Methods. Plenum Press. Nova York, 462p, 1993.
- Hernandez-Blazquez, F.J., Guerra, R.R., Kfoury Jr, J.R., Bombonato, P.P., Cogliati, B., Silva, J.R.M.C., Fat absorptive processes in the intestine of the Antarctic fish (Richardson, 1844). Polar Biology, 29, 831-836, 2006.
- Humbert, W.; Kirsch, R.; Meister, M.F. Scanning electron microscopic study of the oesophageal mucous layer in the eel *Anguilla anguilla* L. Journal of Fish Biology, v.25, p.117-122, 1984.
- Kao, Y.J.; Lichtenberger, L.M. Localization of phospholipid-rich zones in rat gastric mucosa: possible origin of a protective hydrophobic luminal lining. The Journal of Histochemistry and Cytochemistry, v.35, p.1285-1298, 1987.
- Leknes, I.L. Histochemical studies on mucin-rich cells in the digestive tract of the Buenos Aires tetra (*Hyphessobrycon anisitsi*). Acta Histochemica, v.113, p.353-357, 2011.
- Luft, J.H. Ruthenium red and violet. II. Fine structural localization in animal tissues. Anatomical Records, v.171, p.369-415, 1971.
- Lundberg, J.G.; Littmann, M.W. Pimelodidae (Long-whiskered catfishes). In: Reis, R.E.; Kullander, S.O.; Ferraris-Junior, C.J. Checklist of the Freshwater Fishes of South and Central America. EDIPUCRS ed, Porto Alegre, p.432-446, 2003.
- Marchetti, L., Capacchietti, M., Sabbieti, M.G., Accili, D., Materazzi, G., Menghi, G. Histology and carbohydrate histochemistry of the alimentary canal in the rainbow trout *Oncorhynchus mykiss*. Journal of Fish Biology, v.68, p.1808-1821, 2006.
- Murty, V.L.N.; Sarosiek, I.; Slomiany, A.; Slomiany, B.L. Effect of lipids and proteins on the viscosity of gastric mucus glycoprotein. Biochemical and Biophysical Research Communications, v.121, p.521-529, 1984.
- Noaillac-Depeyre, J., Gas, N. Fat absorption by the enterocytes of the carp (*Cyprinus carpio* L.). Cell and Tissue Research, v.155, p.353-365, 1974.

- Ostaszewska, T. Developmental changes of digestive system structures in pike-perch (*Sander lucioperca* L.). *Electronic Journal of Ichthyology*, v.2, p.65-78, 2005.
- Pádua, S.B.; Ishikawa, M.M.; Ventura, A.S.; Jerônimo, G.T.; Martins, M.L.; Tavares, L.E.R. Brazilian catfish parasitized by *Epistylis* sp. (Ciliophora, Epistylididae), with description of parasite intensity score. *Parasitology Research*, v.112, p.443–446, 2013.
- Park, J.Y., Kim, I.S. Histology and mucin histochemistry of the gastrointestinal tract of the mud loach, in relation to respiration. *Journal of Fish Biology*, v.58, p.861-872, 2001.
- Pavanelli, G.C.; Machado, M.H.; Takemoto, R.M. Fauna helmíntica de peixes do rio Paraná, região de Porto Rico, PR. In: A. E. A. M. Vazzoler, A. A. Agostinho & N. S. Hahn (eds.), *A planície de inundação do alto rio Paraná: aspectos físicos, biológicos e socioeconômicos*. EDUEM, Maringá, pp. 301-323.
- Pedini, V.; Dall'aglio, C.; Parillo, F.; Scocco, P. Glycoconjugate distribution in gastric fundic mucosa of *Umbrina cirrosa* L. revealed by lectin histochemistry. *Journal of Fish Biology*, v.66, p.222–229, 2005.
- Pedini, V.; Scocco, P.; Radaelli, G.; Fagioli, O.; Ceccarelli, P. Carbohydrate histochemistry of the alimentary canal of the shi drum, *Umbrina Cirrosa* L. *Anatomia Histologia Embryologia*, v.30, p.345-349, 2001.
- Penha JMF, Mateus LAF. Sustainable harvest of two large predatory Catfish in the Cuiabá river basin, northern Pantanal, Brazil. *Brazilian Journal of Biology*, v.67, p.81-89, 2007.
- Reinecke, M.; Walther, C. Aspects of turnover and biogenesis of synaptic vesicles at locust neuromuscular junctions as revealed by zinc iodide-osmium tetroxide (zio) reacting with intravesicular sh-groups. *The Journal of Cell Biology*, v.78, p.839-855, 1978.
- Rhodes, J.M., Black, R.R., Gallimore, R., Savage, A. Histochemical demonstration of desialitation and desulphation of normal and inflammatory bowel disease rectal mucus by faecal extracts. *Gut*, v.26, p.1312-1318, 1985.
- Sarasquete, C., Gisbert, E., Ribeiro, L., Vieira, L., Dinis, M.T. Glyconjugates in epidermal, branchial and digestive mucous cells and gastric glands of gilthead sea bream, *Sparus aurata*, Senegal sole, *Solea senegalensis* and Siberian sturgeon, *Acipenser baeri* development. *European Journal of Histochemistry*, v.45, p.267–278, 2001.
- Scocco, P., Accili, D., Menghi, G., Ceccarelli, P. Unusual glycoconjugates in the esophagus of a tilapine polyhybrid. *Journal of Fish Biology*, 53, 39-48, 1998.
- Sire, M.F.; Lutton, C.; Vernier, J.M. New views on intestinal absorption of lipids in teleostean fishes: an ultrastructural and biochemical study in the rainbow trout. *Journal of Lipid Research*, v.27, p.81–94, 1981.
- Slomiany, B.L.; Piasek, A.; Sarosiek, J.; Slomiany, A. The role of surface and intracellular mucus in gastric mucosal protection against hydrogen ion. Compositional differences. *Scandinavian Journal of Gastroenterology*. v.20, p.1191-1196, 1985.
- Slomiany, B.L.; Slomiany, A. Secretion of gastric mucus phospholipids in response to b-adrenergic g protein-coupled receptor activation is mediated by SRC kinase-dependent epidermal growth factor receptor transactivation. *Journal of Physiology and Pharmacology*, v.55, p.627-638, 2004.

Sperry, D.G., Wassersug, R.J. A proposed function for microridges on epithelial cells. *The Anatomical Record*, v.185, p.253-257, 1976.

Spicer, S.S., Schulte, B.A. Diversity of cell glycoconjugates shown histochemically: a perspective. *Journal of Histochemistry & Cytochemistry*, v.40, p.1-38, 1992.

Tibbetts, I.R. The distribution and function of mucous cells and their secretions in the alimentary tract of *Arrhamphus sclerolepis krefftii*. *Journal of Fish Biology*, v.50, p.809–20, 1997.

Tocher, D.R. Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science*, v.11, p.107–184, 2003.

Vieira, K.R.I.; Vicentin, W.; Paiva, F.; Pozo, C.F.; Borges, F.A.; Adriano, E.A.; Costa, F.E.S.; Tavares, L.E.R. *Brevimulticaecum* sp. (Nematoda: Heterocheilidae) larvae parasitic in freshwater fish in the Pantanal wetland, Brazil. *Veterinary Parasitology*, v.172, p.350–354, 2010.

Wilson, J.M., Castro, L.F.C. Morphological diversity of the gastrointestinal tract in fishes. In: Grosell, M., Farrell, A.P., Brauner, C.J. (Eds.), *Fish Physiology: The Multifunctional Gut of Fish*. Academic Press, San Diego, p.1–55, 2010.

3.4.7 Figuras e Legendas

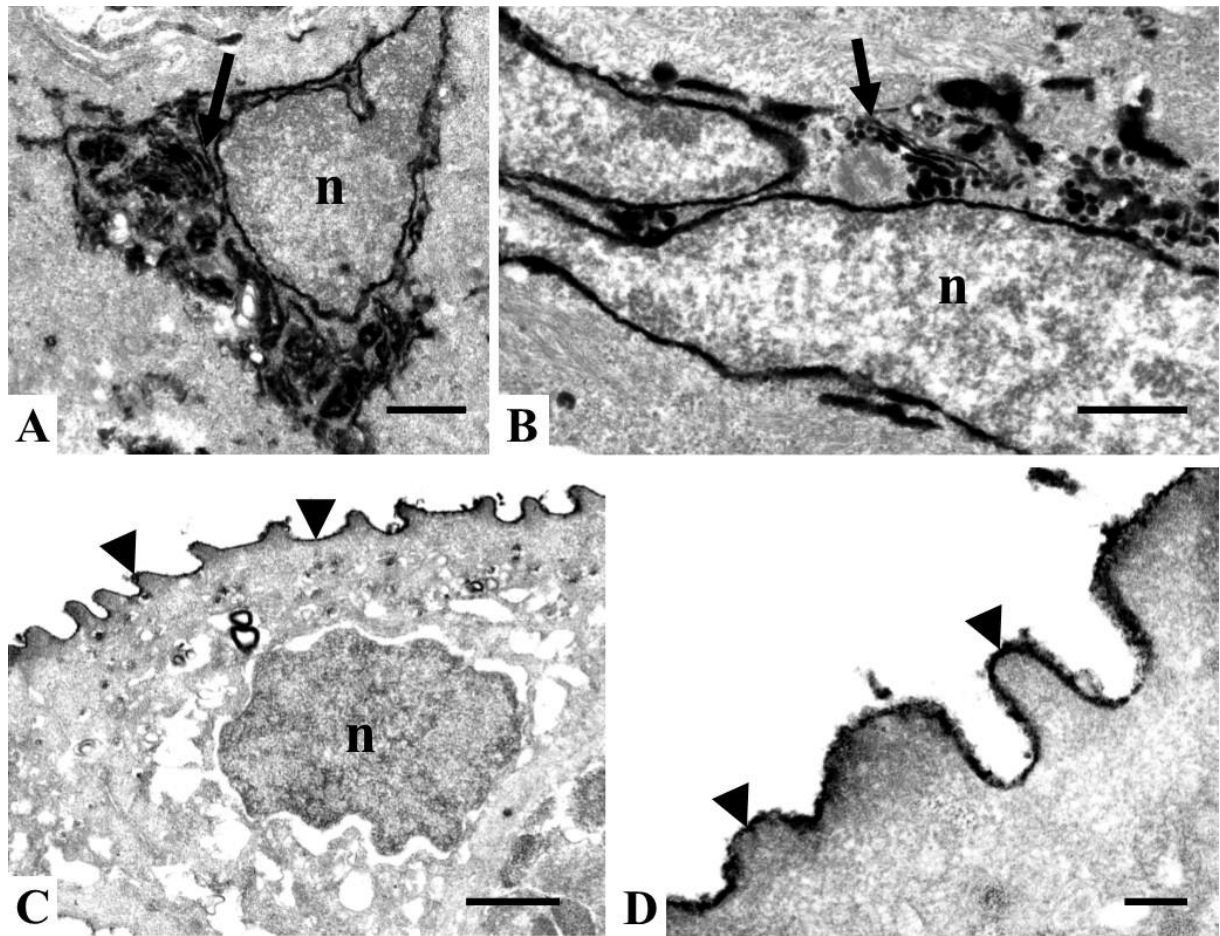


Figura 1. Citoquímica de células epiteliais esofágicas de *H. platyrhynchos*. **A) e B)** Método do tetróxido de ósmio e iodeto de zinco-ZIO para detecção de organelas membranosas, evidenciando marcação no envelope nuclear, em mitocôndrias e no complexo de Golgi. **C) e D)** Detecção de glicoconjugados ácidos, mostrando reação positiva na superfície esofágica. Legendas: n-núcleo, seta preta-complexo de Golgi, cabeça de seta-reação de glicoconjugados ácidos. Barra de escalas: A e B:500nm, C: 1 μ m, D: 200nm.

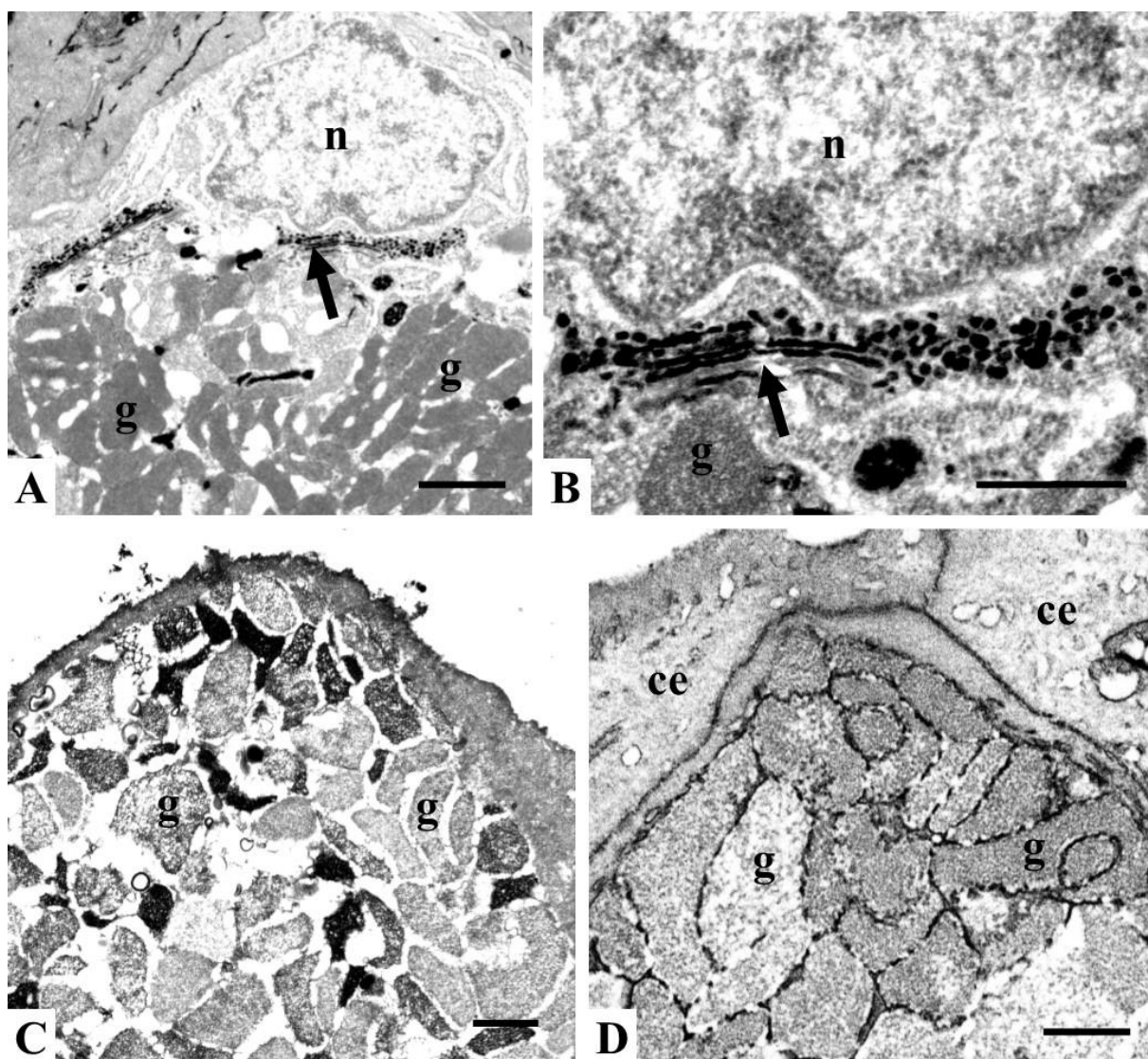


Figura 2. Citoquímica de células caliciformes do esôfago de *H. platyrhynchos*. **A)** e **B)** Marcação eletrondensa no complexo de Golgi com cisternas paralelas, pelo método do ZIO. **C)** Reação positiva para glicoconjugados ácidos, evidenciando grânulos de secreção com eletrondensidade variada. **D)** Marcação para lipídios, notar a presença de revestimento de membrana ao redor dos grânulos. Legendas: ce-célula epitelial, g-grânulos, n-núcleo, seta-complexo de Golgi. Barra de escalas: A: 1µm, B: 500nm, C: 1µm, D: 500nm.

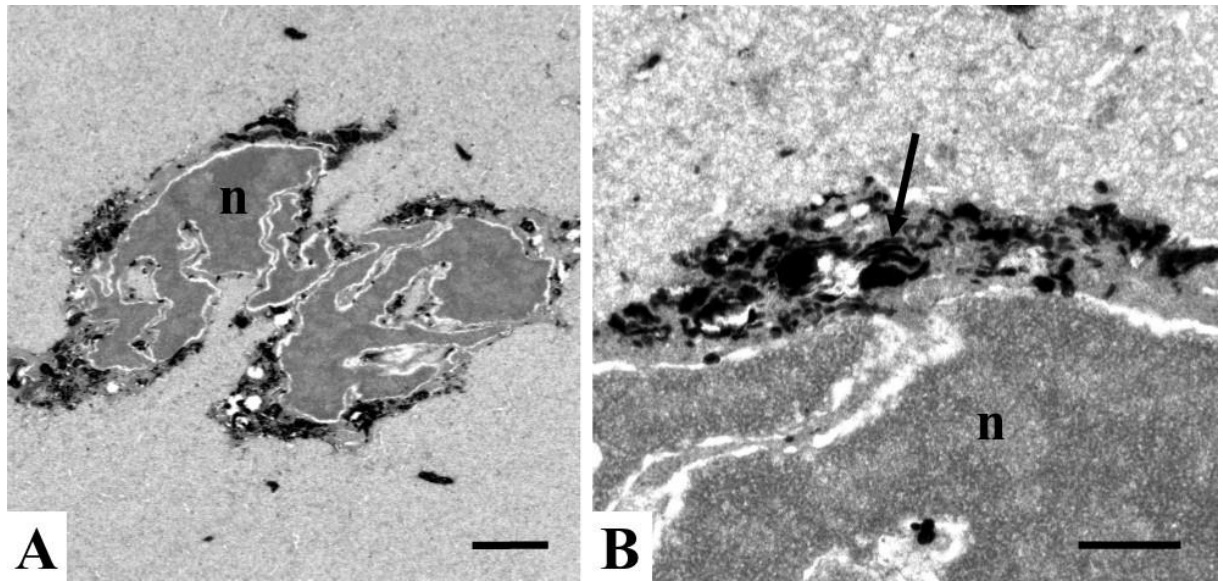


Figura 3. Citoquímica nas células “club” do esôfago de *H. platyrhynchos*. **A)** e **B)** Método do ZIO, evidenciando a presença de poucas organelas membranosas perinucleares. Legendas: n-núcleo, seta-complexo de Golgi. Barra de escalas: A: 2 μ m, B: 500nm.

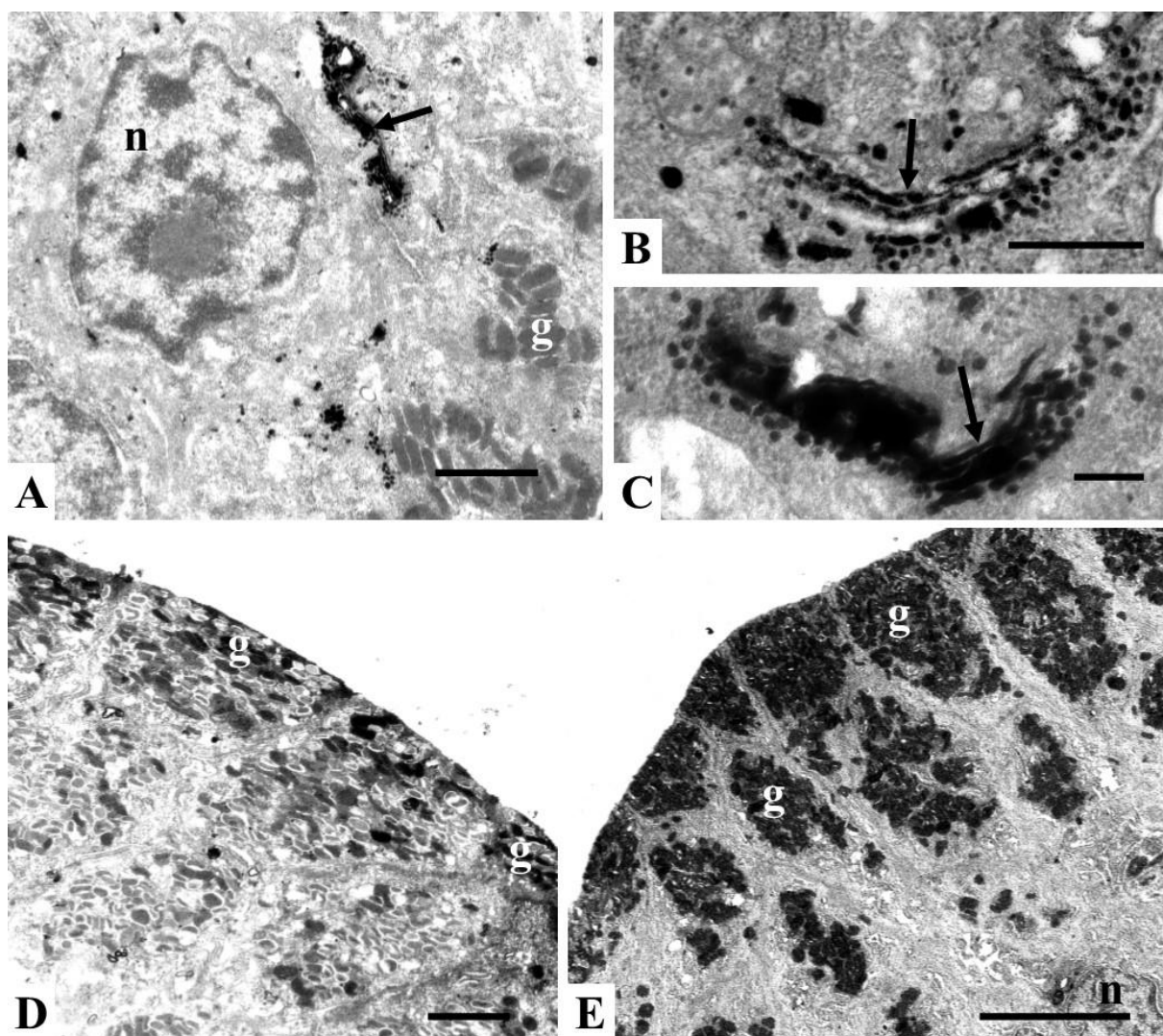


Figura 4. Citoquímica no epitélio gástrico de *H. platyrhynchos*. **A)**, **B)** e **C)** Marcação eletrondensa no complexo de Golgi, pelo método do ZIO. Notar a presença face cis convexa e face trans côncava. **D)** Reação positiva para glicoconjugados ácidos, evidenciando grânulos de secreção com eletrondensidade variada. **E)** Marcação para lipídios, notar que todos os grânulos apresentaram-se fortemente eletrondensos. Legendas: g-grânulos, n-núcleo, seta-complexo de Golgi. Barra de escalas: A: 1µm, B: 500nm, C: 200nm, D: E: 5µm.

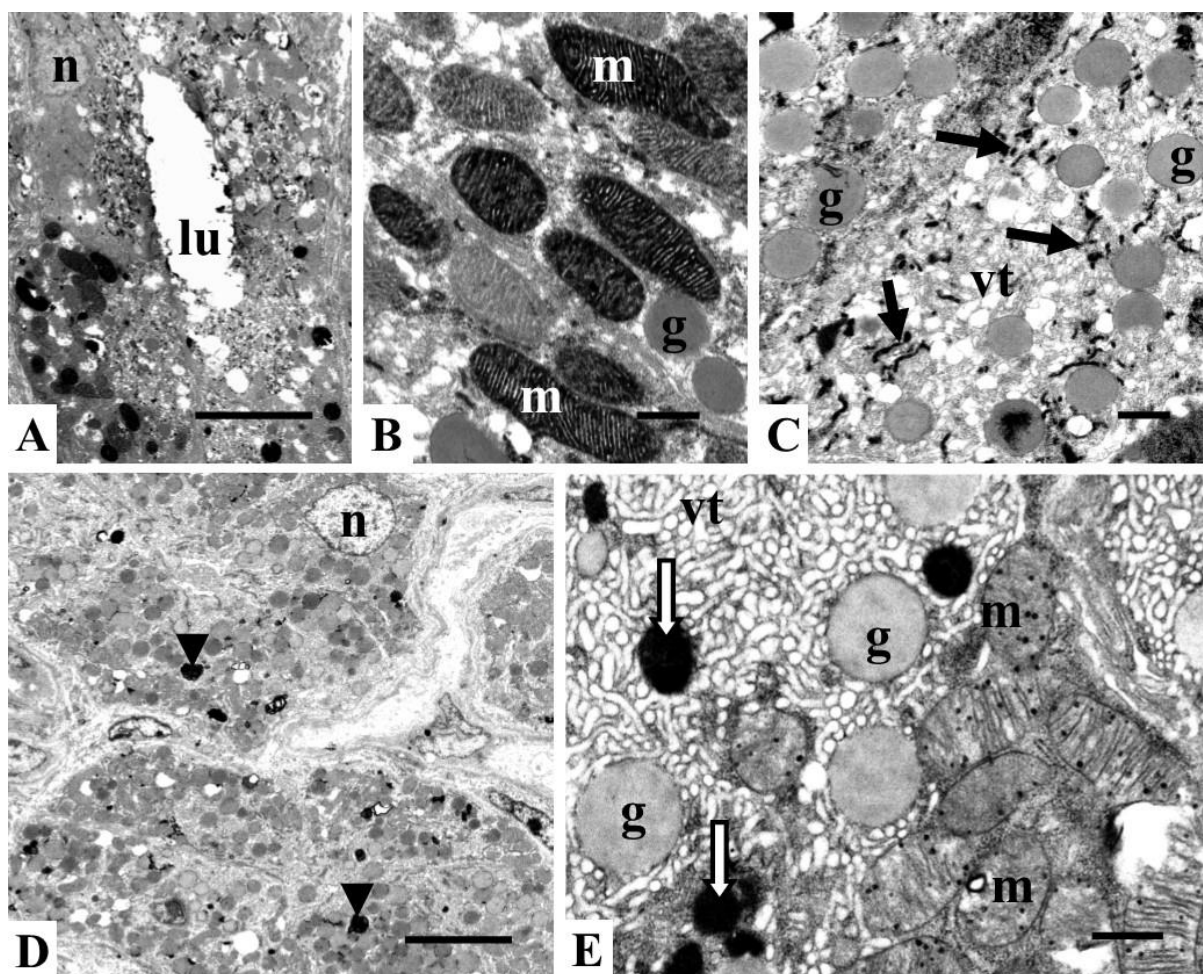


Figura 5. Citoquímica nas glândulas gástricas de *H. platyrhynchus*. A), B) e C) Método do ZIO, evidenciando marcação eletrondensa nas mitocôndrias e em algumas membranas do sistema vesiculotubular. D) Reação positiva para glicoconjugados ácidos, evidenciando grânulos esféricos eletrondensos. E) Marcação para lipídios, notar a presença de gotículas eletrondensas, de marcação nas cristas mitocondriais e em pequenos pontos na matriz mitocondrial. Legendas: g-grânulos de zimogênio, lu-lúmen, m-mitocôndria, n-núcleo, vt-sistema vesiculotubular, seta preta-membranas do sistema vesiculotubular, seta branca-gotículas lipídicas, cabeça de seta-grânulos positivos para glicoconjugados ácidos. Barra de escalas: A: 5µm, B e C: 500nm, D: 5µm, E: 500nm.

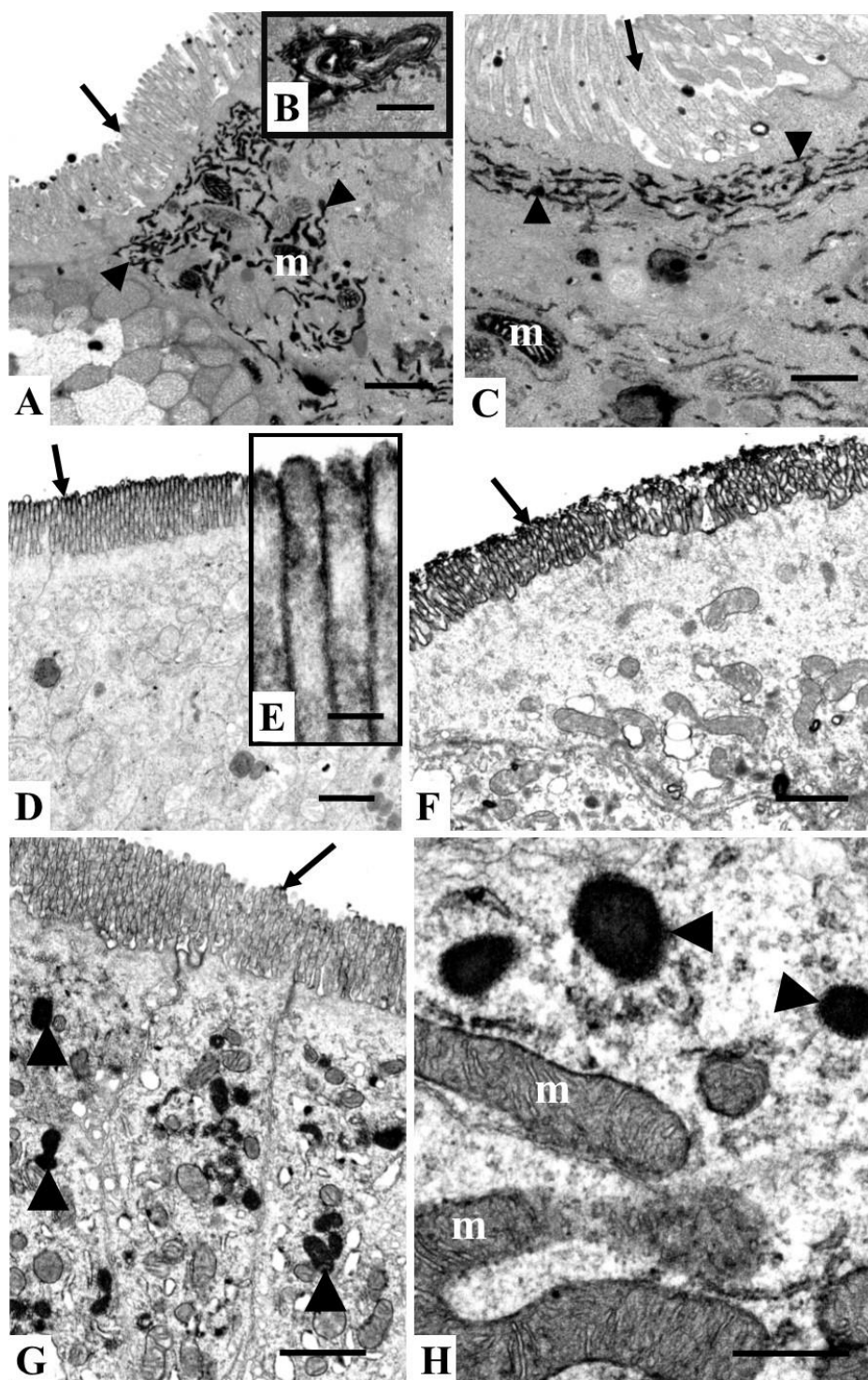


Figura 6. Citoquímica nos enterócitos de *H. platyrhynchos*. **A)** Método do ZIO, exibindo mitocôndrias e retículo endoplasmático liso eletrondensos. **B)** Marcação eletrondensa no complexo de Golgi (método do ZIO). **C)** Predomínio de retículo endoplasmático liso no citoplasma apical, próximo às microvilosidades (método do ZIO). **D), E)** e **F)** Reação positiva para glicoconjugados ácidos ao redor das microvilosidades. Notar a reação moderada no intestino anterior (**D**) e médio (**E**) enquanto que no intestino posterior foi forte (**F**). **G)** e **H)** Marcação para lipídios, evidenciando a presença de gotículas eletrondensas e a marcação nas mitocondriais. Legendas: m-mitocôndria, seta-microvilosidades, cabeça de seta-gotículas lipídicas. Barra de escalas: **A:** 1 μ m, **B** e **C:** 500nm, **D:** 1 μ m, **E:** 100nm, **F** e **G:** 1 μ m, **H:** 500nm.

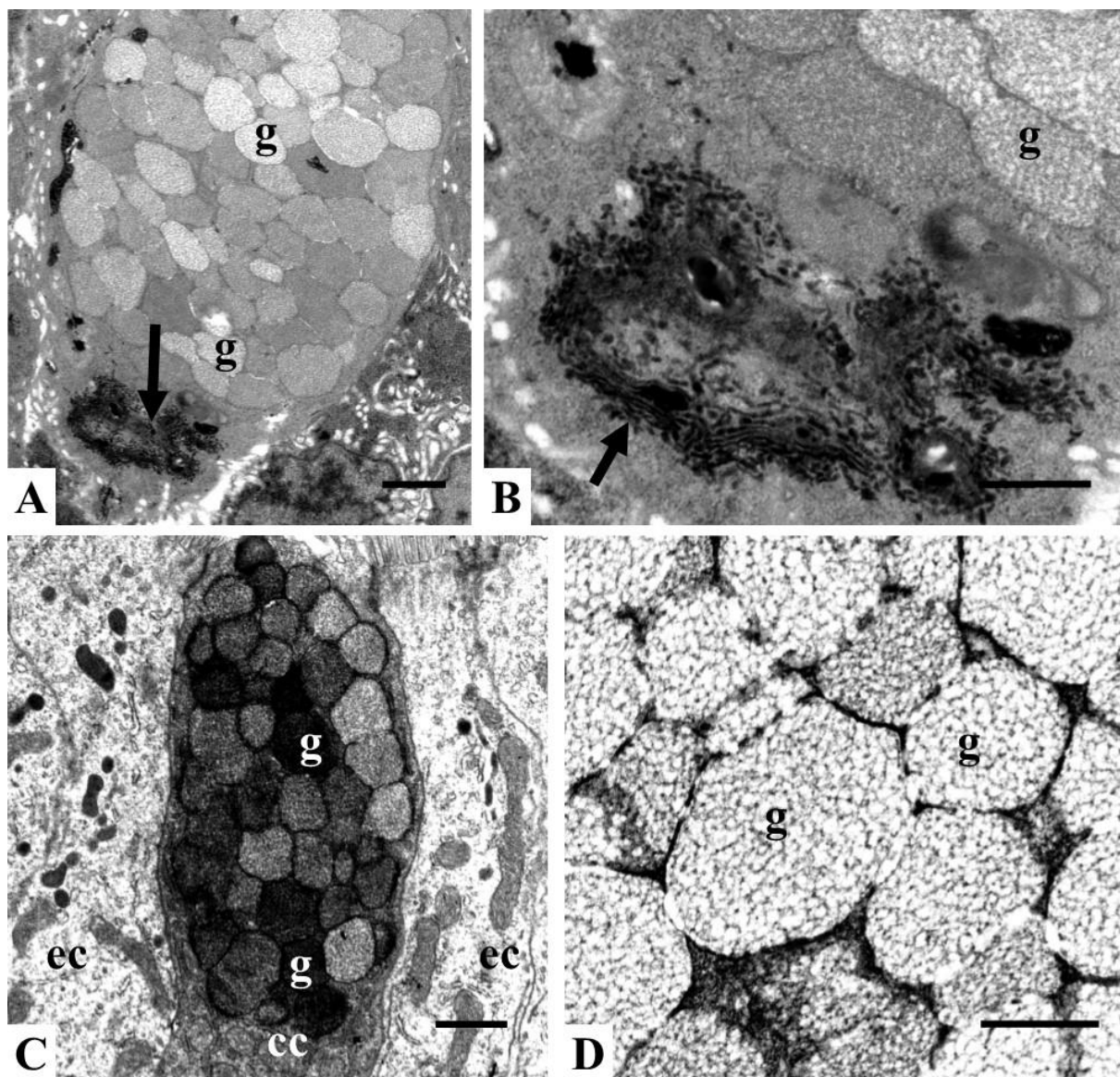


Figura 7. Citoquímica nas células caliciformes intestinais de *H. platyrrhynchus*. **A)** e **B)** Método do ZIO, exibindo complexo de Golgi bem desenvolvido. **C)** Reação positiva para glicoconjugados ácidos nos grânulos de secreção. **D)** Marcação para lipídios, evidenciando a revestimento de membrana ao redor dos grânulos. Legendas: ec-enterócitos, g-grânulos, seta-complexo de Golgi. Barra de escalas: A: 1 μ m, B: 500nm, C: 1 μ m, D: 500nm.

4. Considerações Gerais

Hemisorubim platyrhynchos é uma espécie carnívora que se destaca tanto por sua importância para o funcionamento do ecossistema aquático quanto por seu elevado potencial para cultivo, sendo sua carne muito apreciada devido a qualidade, sabor e ausência de ossos intramusculares – espinhos. Este estudo foi conduzido para compreender a biologia desta espécie e fornecer informações a respeito da morfologia e enzimologia do tubo digestivo para aprimorar as técnicas de criação.

Em ambiente natural, esta espécie alimenta-se de presas inteiras, apresentando um esôfago altamente distensível com pregas longitudinais internas. A camada mucosa apresenta epitélio de revestimento estratificado, com microssaliências superficiais que protegem o epitélio e ancoram mucossustâncias para facilitar a passagem de alimento. Mesmo com esta proteção, as células superficiais estão em constante renovação e para isso apresentam a enzima fosfatase ácida para promover a descamação celular. Numerosas células caliciformes também são identificadas no epitélio de revestimento, secretando grande quantidade de mucossustâncias neutras e ácidas para proteção da superfície epitelial. Ainda assim, em uma possível lesão epitelial, as secreções proteicas das células “club” podem atuar como alerta e promover a defesa do epitélio.

O estômago desta espécie também é um órgão muito distensível e ao receber a presa, promove o início da digestão luminal, por meio das secreções ácidas e enzimáticas das glândulas gástricas, presentes nas regiões cárdica e fúndica. Essas glândulas são compostas por células oxintopépticas e sua alta atividade sintética é refletida na quantidade de mitocôndrias e também na presença de gotículas lipídicas para reserva de energia. A proteção epitelial gástrica é realizada por meio das secreções das células epiteliais, que apresentam grande quantidade de mucossustâncias neutras, mas também mucossustâncias ácidas que protegem o epitélio devido a sua atividade antipéptica. Essas secreções também estão constituídas de lipídios, que são componentes essenciais do revestimento hidrofóbico de proteção do estômago. Ainda, o epitélio gástrico apresentou a enzima fosfatase ácida, para proteção da superfície contra patógenos.

Assim, o esôfago e o estômago apresentaram estruturas para proteção do órgão, para facilitar a passagem do alimento e iniciar a digestão, mesmo de presas grandes. A presença dessas estruturas permite a elaboração de rações compostas por pellets grandes, o que

diminuiria a fragmentação do pellet em contato com a água, reduzindo a perda de qualidade da ração e evitando a queda na qualidade da água.

Após iniciada a digestão proteica no estômago, o bolo alimentar chega ao intestino que por sua vez complementa a digestão luminal, realiza a digestão na borda em escova e a absorção dos nutrientes. O intestino de *H. platyrhynchos* apresentou-se dividido em anterior, medial, posterior e reto. Diversas estruturas descritas neste estudo apontam para o intestino anterior como a principal região de digestão e absorção. Macroscopicamente, as pregas longitudinais são mais desenvolvidas no intestino anterior, bem como, microscopicamente, as microvilosidades são mais longas e apresentam uma maior área de superfície, ampliando o contato do bolo alimentar com as enzimas, tanto luminas como na borda em escova, e aumentando também a superfície de absorção intestinal. Ultraestruturalmente, as células intestinais responsáveis pela absorção, os enterócitos, apresentaram um retículo endoplasmático liso bem desenvolvido, atuando na ressíntese de lipídios absorvidos. As análises de enzimas digestivas também permitiram concluir que o intestino anterior é o principal local de absorção, uma vez que a intensidade da fosfatase alcalina, uma enzima relacionada às membranas celulares onde ocorre transporte ativo e absorção, foi mais forte nesta região. Os dados de comprimento e estrutura do intestino, em especial do intestino anterior, sugerem a necessidade da utilização de uma ração de fácil digestão, uma vez que o intestino é curto e possivelmente a velocidade de trânsito do alimento impedirá a digestão e absorção de componentes de digestão mais lenta.

Ainda, as células caliciformes intestinais foram mais concentradas na região posterior do intestino, atuando para lubrificação e proteção da superfície intestinal, facilitando a passagem do bolo alimentar. Por outro lado, a região retal apresentou poucas células caliciformes, mas este fato é compensado pela característica das células epiteliais que compõe o epitélio retal, apresentando mucossustâncias apicais neutras e ácidas, para proteção e facilitação da eliminação do bolo fecal.

Por fim, no modelo tradicional de classificação do tubo digestivo em peixes, espécies carnívoras apresentam estômago grande e distensível, com muitas glândulas gástricas enquanto espécies onívoras e herbívoras apresentam estômago menor e intestino longo e enovelado. Assim, *H. platyrhynchos* mostrou características marcantes para peixes carnívoros, tornando esta espécie um modelo para estudo de peixes com hábito carnívoro.