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HISTOPATHOLOGICAL CHANGES IN THE ALIMENTARY TRACT OF ALTUM ANGELFISH (*PTEROPHYLLUM ALTUM* PELLEGRIN, 1903) FED WITH MOSQUITO LARVAE (*CULEX* SPP.) IN HIGHLY ACIDIC WATER

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Abstract

Midge larvae, brine shrimp and Culex spp. larvae are important food items for many fin fishes, including Altum Angelfish. Chitin, the unbranched polymer present in the exoskeleton of arthropods, is not easily digestible by all fish species. Its edibility may also depend on variations of environmental factors to which fish are exposed, like acidic water. This is an unusual case of multifocal granulomatous pharyngitis and gastritis in Altum Angelfish, possibly caused by ingestion of mosquito (Culex spp.) larvae and exposure of the fish to highly acidic water (pH below 5.0). The microscopic examination of the larvae fed to the angelfish showed that the foreign bodies which caused the granulomatous reactions were exoskeleton residues of the Culex spp. larvae. However, the effect of acidic water on the integrity of the digestive tract of angelfish and the possible structural changes of the Culex larvae exoskeleton under these circumstances need to be demonstrated.

Key words*: Culex larvae, gastritis, pharyngitis, Pterophyllum altum, water pH.*

INTRODUCTION

Pterophyllum altum (Pellegrin, 1903), also known as Orinoco angelfish, deep angel or Altum Angelfish, is found in the wild in the upper Negro River drainage of the Amazon river basin and in tributaries of the upper Orinoco River (Inírida and Atabapo Rivers) to Puerto Ayacucho, in South America (Barreto-Reyes et al., 2015). These cichlids live in river watersheds where there are moderate amounts of water flow, submerged tree and plant roots and underwater vegetation where they can easily hide from predators. They are more frequently found in very soft and well oxygenated waters, and feed on insects, crustaceans, aquatic plants, small fish and food particles in the water.

Altums are the largest fish species in the Pterophyllum group, reaching 7 inches (18 cm) in length and about 9 inches (20 cm) in height from the tip of the dorsal fin to the tip of the anal fin. In the wild, there are, however, reports on specimens reaching twice the normal size.

The Altum Angelfish can be very challenging to keep in captivity. They are susceptible to stress related diseases, are sensitive to water quality fluctuations, and can be very difficult to breed and feed appropriately. Altums require food with high protein content, several feedings per day, and a strict water change regimen, of at least 25% every week. The water needs to be soft (2-5 dH) and slightly acidic (pH 5.5-6.5).

The paper presents a case of granulomatous pharyngitis and gastritis with intralesional chitinous foreign body in Altum Angelfish kept at a pH lower than 5, and fed food containing live mosquito larvae (*Culex* spp.). It is suspected that the low pH of the water compromised the mucosal integrity of the pharynx and stomach (Mota et al., 2018; Kennedy & Picard, 2012; Colt, 2006; Eshchar et al., 2006; Nagae et al., 2001; Allan & Maguire, 1992; Fromm, 1980) rendering the mucosae penetrable by the non-digestible filamentous chitin present in the exoskeleton of mosquito larvae.

MATERIALS AND METHODS

Eight adult Altum Angelfish were kept in three tanks for breeding purposes, and were fed a combination of midge fly larvae (*Chironomus circumdatus*, Kieffer), mosquito larvae (*Culex* spp.) and brine shrimp (*Artemia* spp.). Reverse osmosis water is used, and water is recirculated through an external canister biofilter.

Seven months prior, the owner administered Tetra Paraguard™, as a preventive measure against parasites. This product is a combination of praziquantel, metronidazole, diflubenzuron and acriflavine. The owner mentioned that, during the time, the pH of the water was maintained at 6.0-7.5. Later, in an attempt to acidify the water, the owner added a mixture of peat (Fluval Peat Granules and Eheim TORF Pellets) in the external filter cannisters, and had made several water changes.

The veterinarian was called to examine the fish because some of them had not eaten for several days, were not breeding, and displayed neurological signs (depression and unresponsiveness). The water was tested with Sera ™ test kits for temperature, pH, total ammonia nitrogen (TAN), nitrite, nitrate and water hardness (KH). The values found are presented in the Table 1, below.

Table 1. Water quality parameters

	Units of measurement	Test results	Suggested optimal range
Temperature	°C	29.1	26-30
TAN	mg/L	5	0
Nitrite	mg/L	0	0
Nitrate	mg/L	0	0
pH	Units	5	5.5-6.5
KH	°dKH	≤ 1	2-4
GH	°dGH	0	2-5

Since it was assumed that the water pH had been fluctuating prior to the sampling, reaching levels below 5.0 due to the use of peat mixture without buffer addition, an experiment with the peat was conducted to determine how acidic it would make the water. In this experiment, Fluval Peat Granules and Eheim TORF Pellets were (separately) finely ground and soaked in Milli-Q® water and tap water, respectively (Figure 1). The samples were shaken for 2 minutes vigorously, left to stand for 5 minutes, and then incubated overnight at 4°C, 24°C and 37°C, respectively (Table 2).

Table 2. Water pH variations with two brands of peat added

Sample	**Milli-Q® Water**			**Tap Water**
Temperature	**4°C**	**24°C**	**37°C**	**24°C**
No peat (control)	-	5.64	-	6.84
Fluval	4.24	4.21	4.05	4.80
Eheim	3.99	3.95	3.95	4.12

The proportions used were: 1.0 g peat/30 ml Milli-Q® water, at an initial pH of 5 and, 1.0 g and 0.25 g peat/10 ml tap water, at an initial pH of 6. A set of controls (no peat added) with Milli-Q® water and tap water (24°C), with pH of 5.64 and 6.84, respectively, was used. Metrohm 691 pH meter was utilized to test the pH.

Figure 1. Fluval Peat Granules and Eheim TORF Pellets

The owner had preserved one deceased angelfish in the freezer, which was then held frozen for two days. After thawing, a partial necropsy was conducted, to allow better perfusion for fixation in 10% neutral-buffered formalin. The fish was held in fixative for 24 hours, and then decalcified for 3 hours in a formic acid solution. Tissues were then routinely processed into paraffin blocks, from which 4 µm sections were cut and stained with haematoxylin and eosin. The skin, muscle, gills, eye, pharynx, heart, spleen, kidney, liver, stomach, intestines and fin were examined using routine light microscopy. Wet preparations of the live food used to feed the fish were also analysed by light microscopy, after fixation in 10% neutral-buffered formalin.

RESULTS AND DISCUSSIONS

1. The peat experiment showed a reduction in the pH below tolerance range in MilliQ® water and tap water (Table 2). The water pH varied with the type of water used in the sample (*i.e.*, MilliQ and tap water), water temperature, and the type of peat used in the experiment. Peat added to Milli-Q® water at 24°C reduced the pH from 5.64 (in controls) to pH 3.95, in the Eheim peat. In tap water samples (initial pH 6.84, in controls) the peat decreased the pH to 4.12 in Eheim peat, and 4.80 in Fluval peat. A severe reduction of pH was recorded in tap water samples (initial pH 6.84) rather than in

Milli-Q® water samples (initial pH 5.64), with 2.72 units (Eheim peat) and 2.04 units (Fluval peat), respectively, versus 1.69 units (Eheim peat) and 1.43 units (Fluval peat), respectively.

2. Histopathological findings in the fish samples. Significant findings were found in the pharinx and stomach of the examined fish. In the proximity of the pharyngeal teeth, in the pharinx mucosa and the mucous glands of the pharinx, there were diffuse granulomatous reactions surrounding golden-brown, refractile, structures. The structures were cilindrical with variable diameters on cross section, or had parallel-sided walls. These bodies were often observed occuring in clusters (Figure 2 and Figure 3).

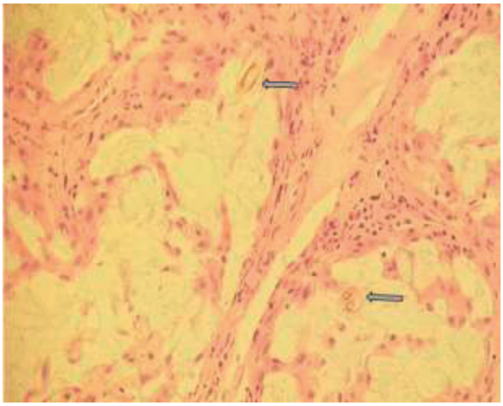

Figure 2. Foreign body granuloma in the mucous glands of the pharynx (arrows). Histological section, H E, 20x

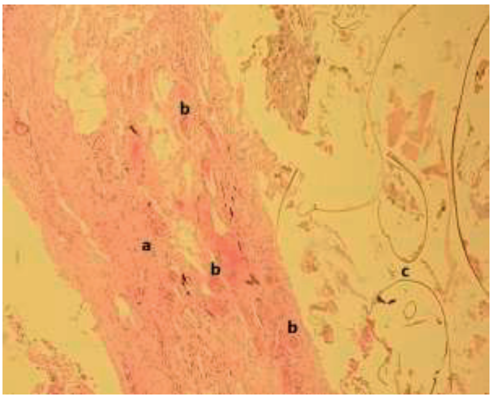

Figure 3. Foreign body granulomatous inflammation in the stomach wall. (a) Hyperplasia. (b) Granulomatous gastritis with clusters of chitin residues. (c) Chitin filaments in the gastric lumen. Histological section, H E, 10x

In the stomach wall, there were similar multifocal granulomatous reactions centred around tube-like, golden-brown structures occuring in bundles or singly, in longitudinal, cross and tangential sections (Figure 3). In the lumen of the stomach there were filaments and fragments of similar material found in the mucosa (Figure 4).

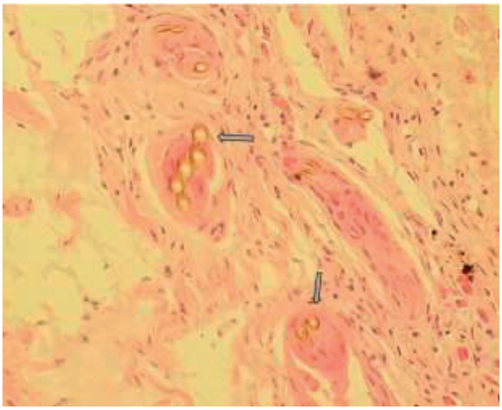

Figure 4. Chitinous foreign body granuloma (arrows) in the gastric epithelium. Histological section, H E, 40x

3. The live food components description: In light microscopy, the body of midge fly larva was as thin, cylindrical and segmented. The posterior end of the body featured short hair-like chitinous filaments (Figure 5).

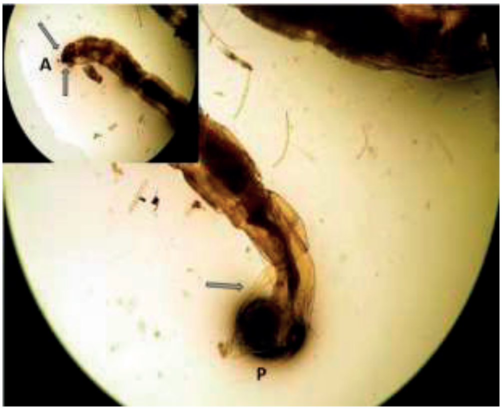

Figure 5. Midge larva external structure. Anterior (A) and posterior (P) ends feature parapods and procerci (arrows)

The brine shrimp exoskeleton appeared thin, covered by short filaments of chitin on the thorax and posterior end of the body (Figure 6).

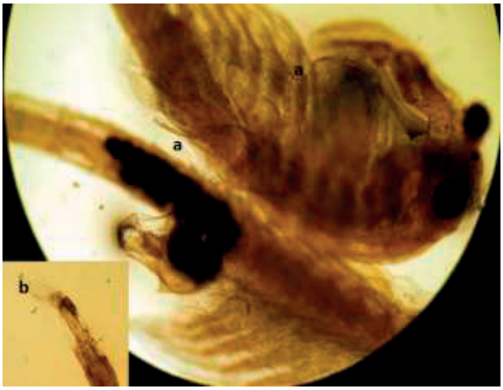

Figure 6. Brine shrimp exoskeleton. (a) Chitinous filaments on torax. (b) Chitinous hairs on the tail

The mosquito larva body had numerous tufts of long hair-like structures on the head, thorax and abdomen. Additionally, there were abundant anal and dorsal brushes and hairs on the posterior end of the body (Figure 7), resembling the structures observed in the pharinx and stomach of the angelfish.

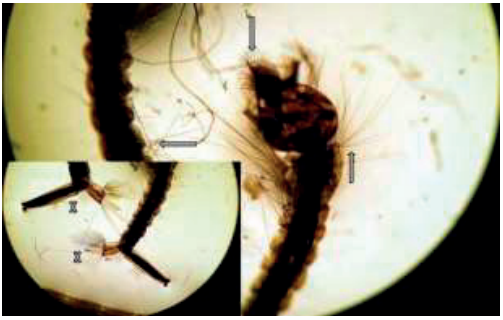

Figure 7. *Culex* spp. larvae exoschelleton. Strands of hairs on the head, thorax and abdomen (arrows). Abundant anal brushes and caudal hairs on the posterior end of the body (X)

Dry commercial, freeze dried or frozen foods often cannot meet all the nutritional requirements for conditioning broodstock, hence supplementation of their diet with live food is common practice (Farahi et al., 2010). Like in the case of *Artemia* (brine shrimp), adult insects and their larvae are largely utilized in fish rearing, being part of the natural diet of fish (Howe et al., 2014; Whitley and Bollens, 2014; Henry et al., 2015). Midge larvae, brine shrimp and *Culex* spp. larvae are recognized as important food items for many ornamental fishes (Patra & Ghosh, 2015). Insects and their larvae are rich in protein, fats, vitamins and minerals (Zamprogna et al., 2017). Artemia has a high nutritive value and conversion efficiency (Kaiser et al., 2003; Farahi et al., 2010). The unbranched polymer chitin is the primary component of the exoskeleton of arthropods (crustacean shells, insect exoskeletons), (Henry et al., 2015). Chitin digestibility varies amongst different fish species due to the variety of chitinase activity (Zamprogna et al., 2017). However, it is highly unusual for finfish to experience the injuries observed in this case from eating the arthropod juveniles and larvae.

Few species of mosquito larvae survive below pH 4.0 (Thamer and Abdulsamad, 2005; Armesto et al., 2017; Clark et al., 2004). We are hypothesizing that the pH of water must have played an important role in the development of the lesions observed in the alimentary tract of the Angelfish fed the live food.

CONCLUSIONS

Following microscopic examination of the feed, it was concluded that the most likely candidate to have caused the lesions seen in the pharynx and stomach was the mosquito larvae, due to its abundant hairs of chitin. It is suspected that the low pH of the water compromised the mucosal integrity of the pharynx and stomach rendering the mucosae penetrable by the non-digestible filamentous chitin present in the exoskeleton of mosquito larvae. It is also possible that the acidic water to have altered the exoskeleton structure of the mosquito larvae, rendering it indigestible to the altum fish. The peat experiment showed that the peat used to correct water pH in the altums tanks may have reduced the pH below the tolerance range. The water could have, at some time, decreased to a pH lower than 4.0, as showed by the peat experiment results. In addition, the pH falling below 5.0 likely resulted in harming the microorganisms in the biofilter, explaining the elevated ammonia. Though, this level of ammonia is unlikely to have caused toxicosis since there would be negligible free-ammonia nitrogen at this low pH. While it is recognizable that the foreign bodies which caused the granulomatous reactions in the stomach and pharynx of the

Angelfish are residues of the exoskeleton of, mainly, the mosquito larvae, the effects of acidic water on the integrity of digestive tract in Angelfish fed live food needs to be further documented. Whether the acidic water may have affected the structure of the exoskeleton, rendering it indigestible by the fish, thus causing the foreign body reactions in the stomach and pharynx, this also needs to be documented further.

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