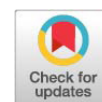


Research Article



Occurrence and Histopathology of *Henneguya* sp. (Cnidaria: Myxozoa) in the Air Breathing Organ of the Sharptooth Fish *Clarias gariepinus* (Clariidae)

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Abstract | For around 49 fish species, respiration is partly ensured by a specific organ called Air Breathing Organ (ABO). ABO replaces fish gills in hypoxic conditions. The present study is dedicated to investigate the occurrence and potential physiological effects due to a myxosporean parasite infecting ABO in *Clarias gariepinus*. To achieve this goal, a total of 339 *C. gariepinus* was investigated for myxosporean infection. Fish sex, size and weight were recorded and light and electron microscopes were used to identify the parasite and to enlighten the physiological impacts. The prevalence was accessed as a function of fish size and climatic seasons. Water physicochemical parameters were collected and its correlation with the prevalence of infection was assessed. The mean prevalence was 10.91% (37/339). No significant difference was observed either between males and females ($\chi^2=0.136$; $df=3$; $p=0.987$) or between seasons ($\chi^2=1.772$; $df=3$; $p=0.621$). However, significant difference was recorded between fish class weight ($\chi^2=11.781$; $df=5$; $p=0.038$). Longitudinal and transverse sections in the ABO revealed cysts establishment in the elastic cartilage of ABO and its cartilage erosion. This leads to limitation of fish's ability to exchange air gases by reduction the folds. Therefore, a crucial attention could be paid to this parasite and develop effective control measures to mitigate its impact on fish health and production.

Keywords | *Clarias gariepinus*, Suprabranchial organ, Prevalence, Electron microscope, Pathology, Physicochemical parameters

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INTRODUCTION

Animal protein contains essential amino acids that are important for a healthy and well-balanced human

diet (Elmadfa and Meyer, 2017). Free-ranging land animals are no longer a significant source of protein, and the production costs of farm-raised animals continue to increase as land becomes more expensive (Guo and Woo,

2009). Fish is a more or less complete food because it contains fat, minerals, oils, and vitamins (Ayanda, 2009), as well as easily digestible proteins (Guo and Woo, 2009). Fish makes up almost 20% of animal proteins worldwide for about 3 billion people and approximately 15% of these same proteins for 4.3 billion people (FAO, 2012). Fish culture is consequently a good option, as production costs are lower over time, representing the best alternative to fill the gap between natural fish catch and the estimated needs of consumers in animal protein consumption. Aquaculture is an important socio-economic sector, especially for rural communities, contributing to livelihoods, food security, and poverty alleviation (Edwards *et al.*, 2002).

Clarias gariepinus Burchell, 1822 (Clariidae) is one of the species possessing a pair of suprabranchial chambers located in the dorsal-posterior part of the branchial cavity, having extensions from the upper parts of the second and fourth gill arches, forming the arborescent organs. This structure is an air-breathing organ (ABO) that could allow this fish species for atmospheric breathing (Teugels, 2011). Beyond the abundant mucus production as a living adaptation in adverse environments (Donnelly, 1973), *C. gariepinus* developed an ability to move between environments and bury itself during drought (Cambray, 2003). Considering their capacity to access abundant oxygen in the air, it appears that fish that breathe atmospheric air grow faster than non-air-breathing species (Lefèvre *et al.*, 2014). Besides, for more decades, Lazard and Legendre (1994) claimed its economic importance. For those reasons, *C. gariepinus* can be cultivated in regular farming conditions or at slightly higher densities (Awe, 2017). Nkamigbo *et al.* (2014) have therefore estimated for two or three times the value of Clarids more than Tilapia's. Therefore, Pasch and Palm (2021) suggested that *C. gariepinus* farming is sustainable and economically profitable aquaculture.

However, *C. gariepinus* could be heavily infected by myxosporean parasites, especially by *Henneguya* species, like any other fish. This requires great care to prevent parasites that can induce diseases and affect its production, particularly in the cases of increased fish farm activities (Michel, 1989; Meyer, 1991; Bondad-Reantaso *et al.*, 2005). The Myxozoan phylum brings together the most common parasites of fish (Schmahl *et al.*, 1989; Feist and Longshaw, 2006). The genus *Henneguya* is the third most diverse in myxozoan species (Lom and Dyková, 2006), and numerous species have been reported infecting wild and farmed fish in many regions around the world (Azevedo and Matos, 2003; Martins and Onaka, 2006; Eiras *et al.*, 2008; Tossavi *et al.*, 2015) with considerable economic impact (McCraren *et al.*, 1975; Yokoyama *et al.*, 2003). Recently, there are reports on myxozoan infection from a diversity of fish species. Gbankoto *et al.* (2015) and Sokolov *et al.* (2019) recorded gonad infection by

Myxobolus sp. and *Henneguya testicularis*, respectively. A high mortality rate was shown in hybrid tilapia infected by *Myxobolus bejeranoi* n. sp. (Lovy *et al.*, 2018). In the cultured *Lates calcarifer*, Brkhanuddin *et al.* (2020) revealed a single henneguyosis infection and attested *Henneguya* is currently a problem for the fish. Moreover, in infected gill filaments, the plasmodia caused swelling or deformation and reduction in respiratory surface area by compression of lamellae (Correya *et al.*, 2021).

In fish, a best localization of *Henneguya* species is gills as the main site of gas exchange in almost all fishes (Dinh-Hung, 2022). Zayed and Mohamed (2004) assured that the gill surface area is greater in the Nile tilapia than in *C. gariepinus*, whereas it can survive for a long period either in the hypoxic water or outside the aquatic environment (Chandra and Banerjee, 2004) due to ABO. As gills, ABO is frequently known to be infested by pathological species of *Henneguya*. Although the process of accurately identifying a *Henneguya* species recorded in ABO is still ongoing, some interesting information has already been gathered using light and electron microscopy to investigate its morphology and the pathology that it produces. The aim of this study was to assess the prevalence of the parasite and its potential damages on the ABO. The correlation of the infection rate of this parasite with the sex and weight of *C. gariepinus* was also investigated.

MATERIALS AND METHODS

STUDY AREA, FISH COLLECTION AND WATER QUALITY

From November 2011 to December 2012, specimens of *C. gariepinus* were bimonthly sampled from Agonlin-Lowé (Adjohoun) located in latitude 06° 39' N and longitude 02° 28' E (Figure 1). It represents the middle sector of the Ouémé valley where the climate is subequatorial with a long wet season (LWS) from April to July followed with a short dry season (SDS) from August to September and a long dry season (LDS) for only October and November and finally the short wet season from December to March. The atmospheric temperature is running from 27.8 to 31.4°C. After collection, fish were then carried to the laboratory. Every fish length, weight and sex were recorded. Physicochemical parameters including pH, temperature (T°C), and Dissolved Oxygen (O₂), were measured *in-situ* using a pH-meter multiparameter pH/Oxi340i/SET. Then, water samples were collected in bottles covered with aluminium foil in order to assess the concentrations for Nitrites (NO₂), Nitrates (NO₃), Ammonium (NH₄), orthophosphate (PO₄), and suspend solids (SS).

PARASITE OBSERVATION, HISTOLOGY AND ELECTRON MICROSCOPY ANALYSIS

Fish were euthanized using Tricaine methanesulfonate (MS-222) for dissection (Anon, 2020). The ABO was

delicately removed from the dorsal-posterior chamber of the branchial system. The cysts were carefully cut from the organ and placed in a saline solution. When a cyst burst, a drop of fresh myxospores was observed using the light microscope (Optim Microscope Jeulin, model n° 571 006) and photographed. Based on the spore morphology and morphometric data including polar capsule length and spore total length and spore body length recorded following Lom and Arthur (1989), tissue tropism other available species, we concluded parasite has not been appreciated as a novel species. It should be recognized as *Henneguya* sp. as the molecular techniques will be employed.

with a CI equal 95%.

$$P = \frac{\text{Number of infected fish}}{\text{Total number of fish examined}} \times 100$$

RESULTS AND DISCUSSION

INFECTION RATE AND WATER QUALITY

This research on *Henneguya* sp. infection reported a prevalence equivalent 10.91%. The maximum seasonal prevalence was recorded in LDS while the minimum was obtained in LWS. Females specimens seemed to be more infected than males in SDS and SWS. There was no prevalence according to sex upper to 14% while the it was the lowest in LWS (Figure 2). Significant difference ($\chi^2 = 0.136$; $df = 3$; $p = 0.987$) was observed neither in the prevalence between males and females (Figure 2), nor between seasons ($\chi^2 = 1.772$; $df = 3$; $p = 0.621$). Whereas, the occurrence of *Henneguya* sp. has decreased from fish in the largest weight class to fish in the smallest weight class, from around 20% to 5% (Figure 3). A significant difference was recorded ($\chi^2 = 11.781$; $df = 5$; $p = 0.038$).

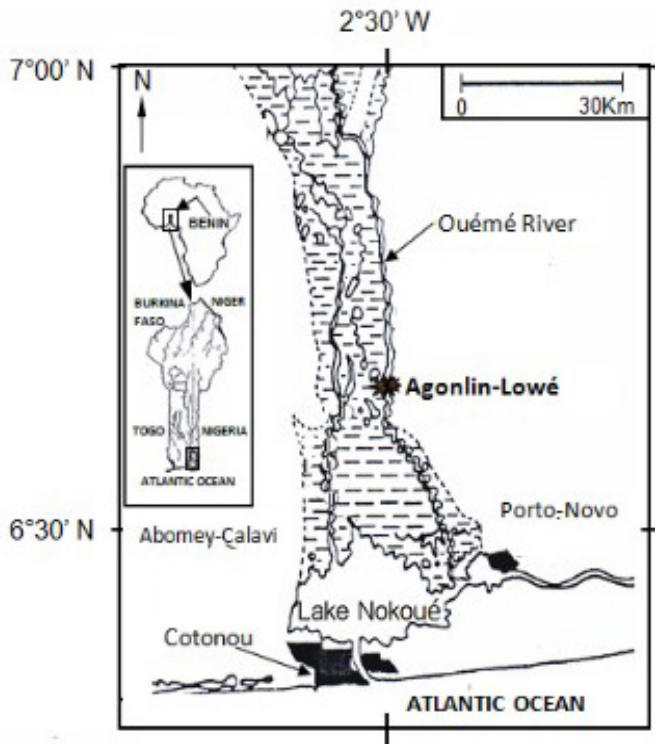


Figure 1: Map of the study area showing Adjohoun (Agonlin-Lowé) on the Ouémé riverbank in Benin (West Africa).

Any smears containing spores were stained with May-Grünwald Giemsa solution. Then, the smears were secured using Canada Balsam medium. The process for histological analysis and transmission microscopy are as described by Tossavi *et al.* (2015).

DATA ANALYSES

The prevalence of infection was determined according to climatic seasons following the formula below. The probable influence of the water quality on the prevalence was assessed using the Pearson correlation coefficient. The chi-square test (χ^2) was used to prove the variability between male and female infections on the one hand and between host sex and climate seasons on the other hand. The statistical analyses were carried out using the Minitab 18 Demo software; difference were considered significant

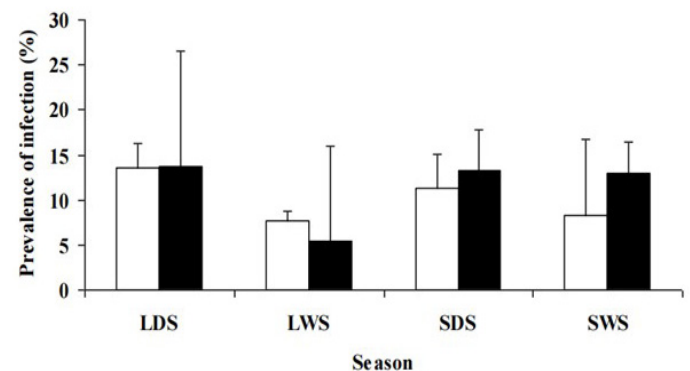


Figure 2: Prevalence of *Henneguya* sp. in *Clarias gariepinus* (female = filled bars and male = open bars) from Agonlin-Lowé in the delta of Ouémé River (Bénin, West Africa). Sampling season given on x-axis (LDS = long dry season, LWS = long wet season, SDS = short dry season, SWS = short wet season).

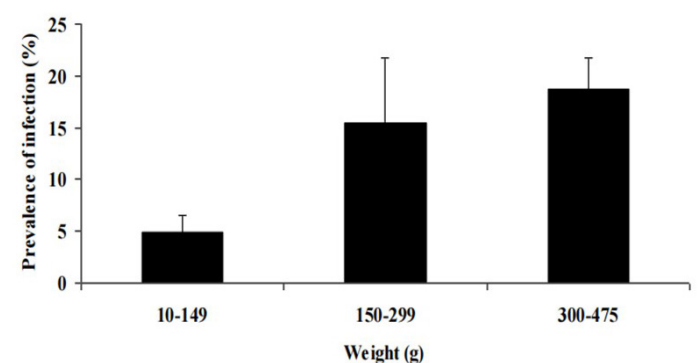


Figure 3: Prevalence of *Henneguya* sp. infection in *Clarias gariepinus* from Adjohoun (Agonlin-Lowé) in the Ouémé River. Classes of weight (g): x-axis.

The data shown in Table 1 refer to the physicochemical parameters measured during this study. No significant variations were detected. Furthermore, no significant difference was reported between the total prevalence of *Henneguya* sp. and the mean value of the physicochemical parameters, except that a negative influence was perceived because of NH₄ (Table 2).

Table 1: Physicochemical parameters of Adjohoun (Agonlin-Lowé) in the ouémé river and seasonal prevalence of *Henneguya* sp. Tossavi *et al.* (2015), Amended.

Water parameters	LDS	LWS	SDS	SWS
T°C	30.55	28.85	26.5	27.7
pH	7.26	7.05	7.2	7.56
O ₂	3.24	1.54	2.78	3.46
NH ₄	0.29	1.03	0.98	0.44
NO ₂	0.25	0.04	0.17	0.02
NO ₃	1.1	5.79	17	1.1
PO ₄	0.04	0.36	1.28	0.57
SS	27	51.5	59	40
PREV	13.69	6.66	12.09	10.44

LDS: Long Dry Season; LWS: Long Wet Season; SDS: Short Dry Season; SWS: Short Wet Season; PREV: Prevalence expresses as percentage.

Table 2: Pearson correlation between prevalence and water physicochemical parameters in Adjohoun (Agonlin-Lowé).

	T°C	pH	O ₂	NH ₄	NO ₂	NO ₃	PO ₄	SS
r	0.138	0.355	0.801	-0.619	0.808	0.041	0.055	-0.469
p	0.862	0.645	0.199	0.381	0.192	0.959	0.945	0.531

r: Coefficient of correlation; p: Probability.

MYXOSPORE DESCRIPTION

The study found an average of 9 (3 to 17) cysts per infected fish. The cysts were soft and yellowish or whitish coloured with a round or spherical shape. Cysts diameter was about 2 to 6 mm. The cysts were found attached to the gill arches (a), specifically at the tip of the bulb of the ABO (Figure 4b) and sometimes along the extension of the mean stem (Figure 4c).

Mature spores were viewed elongated (Figure 4d). The total length of the spores was 36.8 (3 2 to 41.7) µm whereas the length of the valvular cell was 13.7 (13.1-16) µm and its width was 4.9 (4-7) µm. The spores have two elongated and equal-sized polar capsules that tapered at the anterior, occupying about half of the spore. The spore body was fusiform in front view and had a thin sutural line joining the two valves (Figure 4e, f). The surface of the valves was smooth and had two thin and equal caudal processes measuring from 16 to 21.5 µm in length. In valvular view, the spores presented a protruding anterior rostrum (Figure 4e, f).

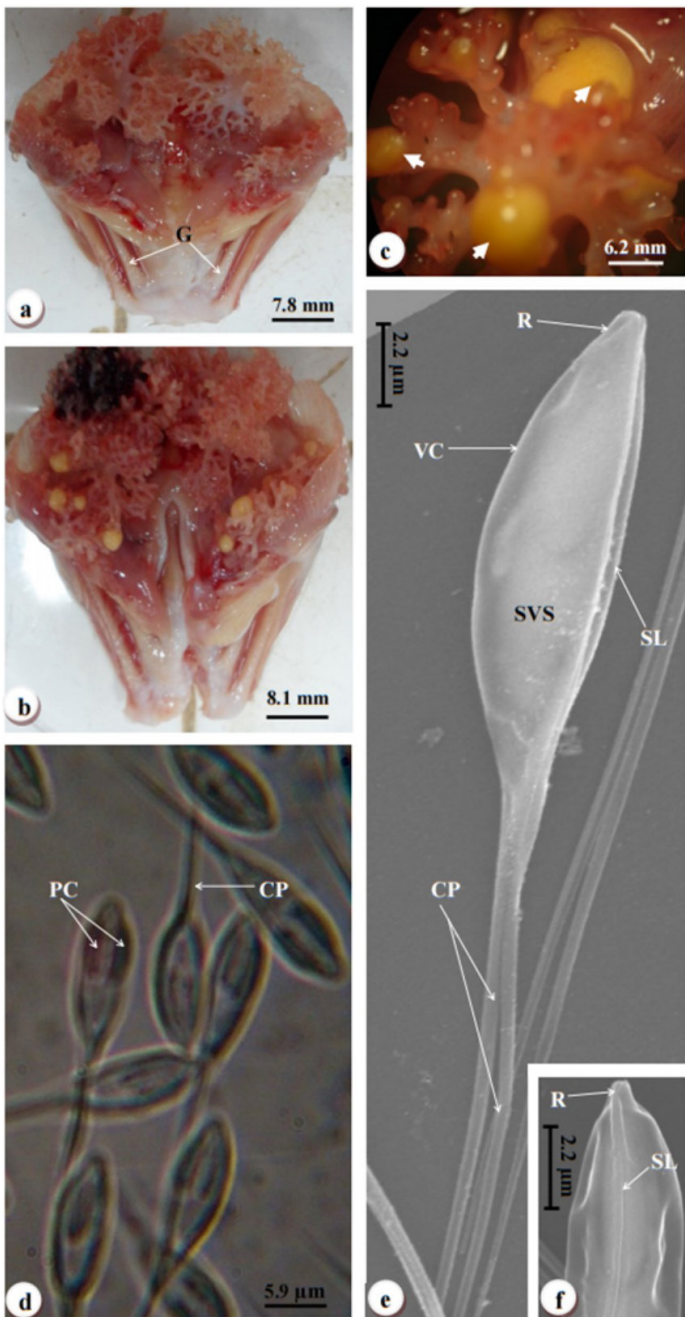


Figure 4: Digital camera photographs illustrating various aspects of the ABO infection. (a) Uninfected ABO demonstrating organ support by the gill arches (G). (b) Infected ABO displaying multiple cysts (thick arrowheads) at the bulb's tip. (c) Infected ABO showcasing large yellowish cysts (white arrowheads) occupying the tip of the bulbs and the stems of the ABO. (d) Photomicrograph of fresh myxospores of *Henneguya* sp., revealing the spore body (Sp), two pyriform polar capsules (PC), and the caudal processes (CP). (e) Scanning electron microscope photomicrographs of the myxospores. (f) Close-up highlighting the smooth valvular surface (SVS) of the myxospore, the sutural line (SL), and the caudal processes (CP) extending from the valves, as well as the rostrum (R) extending from its anterior end. (g) Profile view of the anterior end of the myxospore, clearly showing the sutural line (SL) and the rostrum (R).

HISTOPATHOLOGICAL STUDIES

The study carried out transmission electron microscopic observations on the ABO regarding to understand enough the host-parasite interaction. The ABO was found to consist of a dense connective tissue surrounding elastic cartilage in which myxospores were established (Figure 5a). For healthy ABO, anatomic structures like folds and intervening spaces were observed in the elastic cartilage (Figure 5b), whereas in infected ABO, these structures were progressively destroyed as the myxospores have grown and multiplied (Figure 5c). This resulted in the complete disappearance of the folds and intervening spaces, leaving a clear field for the myxospores (Figure 5c, d). A section in the infected bulb confirms the damage and progression of destruction of the cartilage until it becomes completely loose (Figure 5d).

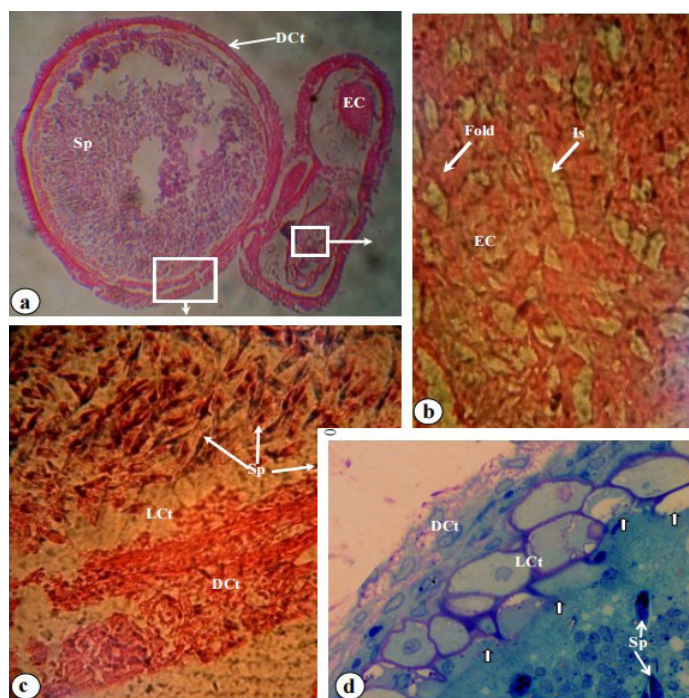


Figure 5: Photomicrographs of different sections from the tip of the ABO bulb, stained with Haematoxylin-Eosin and toluidine blue. (a) Transverse section of an infected bulb tip, stained with Haematoxylin-Eosin, revealing a dense connective tissue (DCt) and elastic cartilage (EC) filled with myxospores (Sp) of *Henneguya* sp. (x 400). (b) Haematoxylin-Eosin stain of a transverse section in the elastic cartilage (EC) of an uninfected ABO, showing large folds and intervening spaces (Is) (x 600). (c) Haematoxylin-Eosin stain of a transverse section of an infected bulb tip, illustrating the dense connective tissue (DCt), loose connective tissue (LCt), and elastic cartilage (EC). Myxospores were observed within the elastic cartilage (EC) and the LCt (x 600). (d) Toluidine blue stain photomicrograph of an infected bulb tip section of the ABO, demonstrating myxospores (Sp) within the elastic cartilage (EC). Notice the progressive destruction (thick white arrow) of the elastic cartilage (EC) towards the loose connective tissue (LCt) (x 1,000).

Transverse sections of the ABO observed by transmission electron microscopy showed an external thick layer of dense connective tissue covering the loose connective tissue (Figure 6a). The elastic cartilage was separated from the loose connective tissue by a thin layer of fibrils known as the perichondrium (Figure 6b, c). The parasite-host interface showed the presence of a few mature myxospores in the elastic cartilage, with the perichondrium almost eroded (Figure 6c). In fact, it had completely disappeared in some areas, leaving wide empty spaces in the elastic cartilage (Figure 6d).

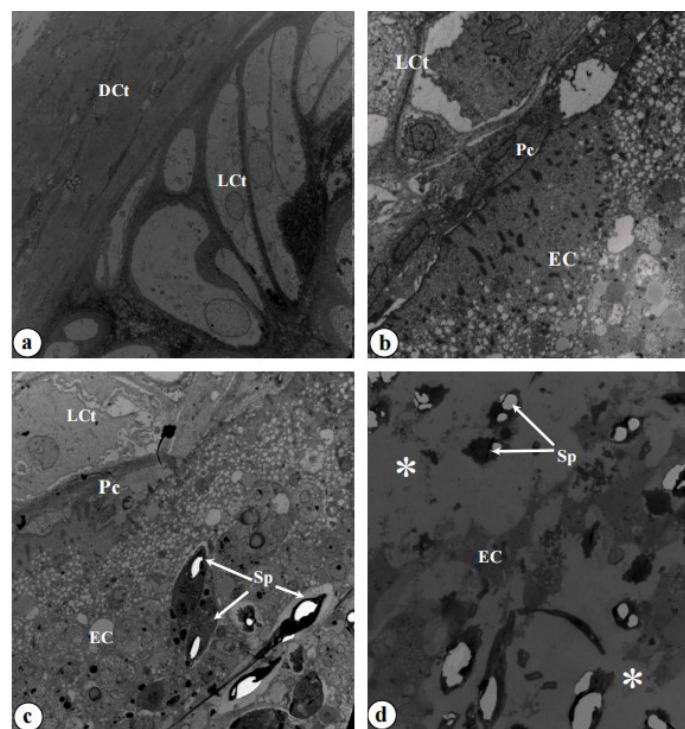


Figure 6: Transmission electron microscope photographs of the tip of the ABO bulb. (a) Section displaying the dense connective tissue (DCt) covering the loose connective tissue (LCt) (x 2,500). (b) Section revealing the loose connective tissue (LCt) and the elastic cartilage (EC), with the perichondrium (Pc) separating the loose connective tissue (LCt) from the elastic cartilage (EC) (x 2,000). (c) Parasite-host interface demonstrating myxospores (Sp) within the elastic cartilage (EC), with the perichondrium (Pc) undergoing significant damage (x 2,200). (d) Section of the elastic cartilage (EC) containing myxospores (Sp), displaying cellular destruction within the elastic cartilage (EC) and empty spaces (white stars) (x 2,500).

PARASITE IDENTITY AND INFECTION RATE ASSOCIATED TO ENVIRONMENTAL QUALITY

According to Molnár *et al.* (2002), only zoological methods are not enough to determine the validity of morphologically similar myxospores, which are also likely to have an affinity for the same tissues and taxonomically closely affiliated host species. As suggested by Adriano *et al.* (2009), this same concept can be applied to morphologically similar

myxosporea found infecting different organs in the same host species, since the site of infection cannot be used as a taxonomic criterion. To overcome the limitations of morphological identification of the studied species, it will be considered as *Henneguya* sp. as a precaution until research its molecular identification will be provided.

According to the literature, a prevalence of 10.91%, provided from the present work, was the lowest recorded from ABO (El-Mansy and Bashtar, 2002; Reed *et al.*, 2003; Abdel-Baki *et al.*, 2011; Morsy *et al.*, 2012). Moreover, Gbankoto *et al.* (2001), Milanin *et al.* (2010), and Barassa *et al.* (2012) pointed out no sex influence on the prevalence. However, Muzzal (1995) and Gbankoto *et al.* (2003) reported a significant difference in prevalence between host sexes. Regarding insignificant seasonal prevalence similar results were shown with other gill myxosporean parasites in Benin (Gbankoto *et al.*, 2001).

El-Mansy and Bashtar (2002) suggested that the highest infection rate was associated with a decrease in length and weight of the fish. The present results are rather in accordance with Mitchell (1988), who reported that parasite infection was high in adult fish. However, those conclusions should be based on the length characterization of the studied sample. Molnár (1998) also recorded the highest prevalence of *Henneguya creplini* Gurley, 1894 in pikeperch (*Stizostedion lucioperca*) larger than 40 cm.

The physicochemical parameters were not correlated with the seasonal prevalence. Also, Tossavi *et al.* (2015) have made the same conclusion regarding intestine infection in *C. gariepinus*. It could be suggested that physicochemical parameters minimized the environmental effect on *C. gariepinus* in the culture system (Lefèvre *et al.*, 2014) opposing Ernst *et al.* (2005) and Khidr *et al.* (2012), who attested that seasonal prevalence of fish parasites was supposed to be influenced by environmental factors. Therefore, the influence due to NH₄ could be justify with Narr *et al.* (2019) who demonstrated the effects of parasite load and prevalence based on the presence of particulate nutrients. However, it was important to note that insufficiently aerated water and combined use of low-quality feed and poor water exchange leads to high organic and hence microbial loading (Lefèvre *et al.*, 2011) in rearing conditions. Pollution and poor environmental conditions which often address fish immunity making them more susceptible to parasitic and diseases (Hecht *et al.*, 2007). More specifically, due to the intermediate host, farming in ponds or fissured basins exposes fish to myxosporidial infections.

HISTOLOGY AND POTENTIAL DAMAGE PATHOLOGY

In contrast to regular cases of myxosporidian infection, the histological analysis revealed that the development of

Henneguya sp. did not occur in the wall of the ABO. As the cysts were established in the core of the bulb end, it has demonstrated the affinity of the parasite to the tissue. The cyst was shown to contain immature and sporogenic stages at the periphery, whereas its centre was filled with mature myxospores, as suggested by El-Mansy and Bashtar (2002) and Abdel-Ghaffar *et al.* (2008). The increase in cyst size and maturation of spores weakned the interspaces, which were subsequently destroyed, along with the folds. As concerned, the parasite has lodged in the tuberculae eating the interspaces and progressed towards the superficial surface. A similar mode of degradation has been observed in the fish with *Henneguya* developing in the inner wall of the intestine epithelium where destruction started before going to the circular muscle (Tossavi *et al.*, 2015). This led to severe damage and deformations of the endothelial cells lining the elastic cartilage of the ABO cells. In the same way, Adriano *et al.* (2005), who reported the thickness of the externa tunica and the granulomatous layer around the cyst. Whatever it is, Banerjee (2007) have shown dilatation of blood vessels, which degenerates into haemorrhage and respiratory failure, as in the case of proliferative gill sphaerodiosis.

According to Teugel (2011), *C. gariepinus* depends more on atmospheric O₂ than most facultative fish air-breathers like an obligate air-breather (Das, 1927; Singh and Hughes, 1971) although it was shown that its gills are less efficient in extracting O₂ from the ventilator water current (Fernandes *et al.*, 1994). The establishment of the cyst in the cartilaginous tissue of the ABO in catfish is responsible for serious lesions in the elastic cartilage, leading to respiratory functional disorders, loss of appetite, and weight (El-Mansy and Bashtar, 2002; Adamek-Urbanska *et al.*, 2021). For these reasons, any infection occurring in the ABO is a substantial phenomenon of reducing gas exchanger surface. Given that *C. gariepinus* is an important farmed fish worldwide, it is possible that if the parasite became established, it could have a serious impact on fish health.

It is important to access a large knowledge of the ultrastructure of the cyst wall (Current and Janovy, 1976) in order to understand the stress due to the pathogenicity of the parasite on its host. In this study, the cyst wall border was not observed. This is in disagreement with other myxosporians described by El-Mansy and Bashtar (2002). This situation has made the destruction of the ABO internal structure evident which has rapidly gone through the perichondrium closed to the loose connective tissue. Furthermore, the entire ABO, including an internal cartilaginous core, implies additional support that prevents the collapse of the respiratory lamellae when the fish faces the loss of water. The respiratory surface is then freely exposed, so that the fish can continue aerial exchange of gases for fairly long periods (Ahmed *et al.*, 2008).

In ponds, myxosporean infections are the most common causes of mortality, thereby affecting fish production drastically. The presence of cysts could never represent a good motivation to enhance gill functions. ABO infection by *Henneguya* sp. and drastically reduces the respiratory surface. As experimented by [Zaccone et al. \(2018\)](#) with other air-breathing fishes, it may be important to investigate the implication of neuroepithelial cells in this phenomenon.

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NOVELTY STATEMENT

The research provided details in the possible destruction capability of a *Henneguya* species on the air breathing organ of *C. gariepinus* species associated to its prevalence and influence from water physicochemical parameters.

AUTHOR'S CONTRIBUTION

TND: Designed the study, collected samples and data in laboratory, carried out the transmission electron microscope observation, prepared figures and drafted the manuscript.

SM: Assisted in samples collection and parasite fixation and prepared tables.

ANF: Assisted for sample collection, for data curation and revised the manuscript.

EML: Supervised the TEM observations, and critically revised the manuscript.

GA: Co-designed the study and critically revised the manuscript.

All authors contributed to improve the quality of the final manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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