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Research Article

Endogenous Factors Associated To Anatomo-Pathological Lesions of Cultured Fish Species in the West Region of Cameroon

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ABSTRACT

The study of anatomo -pathological lesions carried out on fish cultured and delivered for human consumption is essential. These lesions are an indication of pathologies which can negatively impact human health status. A study was therefore carried out on 2254 farmed fish specimens collected in the West Region of Cameroon, for better understanding of the diseases affecting fish and improvement of their production. Macroscopic and histological examination of fish specimens helped determine the prevalence of various anatomical pathologies or abnormalities in various parts of the body namely haemorrhagic lesions (19%), erosion (18%), body deformities (1.1%), colour changes (0.6%), exophthalmia (0.2%) as well as liver (1.3%) and gonad (2%) abnormalities. Fish species and sex did not have a significant influence on the prevalence of the pathologies. The parasite prevalence was insignificantly correlated with the observed abnormalities. Morphological and histological alterations could be used as biological markers for degradation of environmental conditions and fish quality. Additional studies should be undergone for a better understanding of the various causes of fish pathologies and their consequences on fish farming and production.

Keywords: Cameroon, Farmed fish, Productivity, Lesions, Prevalence.

INTRODUCTION

Aquaculture is important for food security, livelihood, nutrition, and socioeconomic well-being in many countries around the world. Fish production through aquaculture provides a safe and reliable source of fish for human consumption [1-3]. According to Alves LM et al, fish products are sometimes subjected to various diseases which can induce anatomo-pathological lesions [4]. This situation results in a change in their organoleptic characters and predisposition of humans to toxin infections and zoonoses. These lesions can be macroscopic (hemorrhages, erosions, deformations, etc.) and/or microscopic (gonadic, hepatic and ocular abnormities) [5,6]. The suspicion of an affected fish is usually followed by an external clinical examination. However, the symptoms of infected fish are not clear from clinical observation and macroscopic lesions, hence the interest of histological sections. The pathogens involved in these lesions feed by decomposing the tissues of the organism [7]. Several works related to fish anatomo-pathological lesions have been carried out, notably in Algeria and in Quebec but none in Cameroon [5,8-11]. This study aimed to assess the health status of reared Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*) through identification and description of various lesions observed and probable risk factors (fish species, etiological agents).

MATERIALS AND METHODS

Study area and period

The study was carried out from December 2018 to December 2019 in three administrative divisions (Menoua, Noun and Hauts-plateaux) of the West Region of Cameroon (9°50' – 10°20' E

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and 5°10' – 5°40' N) (Figure 1). The West Region has a typical sudano-guinean climate, characterized by a short dry season (mid-November–mid-March) with a temperature range of 20 °C– 27°C and 16°C–23°C respectively. The average annual rainfall is 1600 mm and relative humidity ranges from 49 –97.9% between the dry and rainy season [12].

Figure 1: Map of Cameroon showing the West Region of the administrative Divisions and sub-divisions with study sites within Menoua. Noun and Hauts-plateaux Divisions. (Adapted from National institute of Cartography. 2019). **Note:** ( )-Study area; ( )-Boundary of subdivision; ( )-Boundary of division; ( ) -Boundary of region.

Sampling of fish: On each farm, fish were selected using the simple random sampling method. Overall, Nine fish farms were selected based on an agreement with their owners. The sample size was determined according to Thrusfield M et al, as follows [13]:

N= (p(1-p)z^2)/e^2

Where, N: size of the base sample; Z: 1.96, level of confidence (1-α)=95%; e: margin of error=5%; p: Prevalence (50% of prevalence was used for this purpose).

Fishes were captured live with nets or after emptying the pond. Fish collected as earlier described by Ngueguim DF et al, consisted of *Clarias gariepinus* (692), *Cyprinus carpio* (593) and *Oreochromis niloticus* (969). Fish samples were transported during the early hours (9:00-10:00) of the day in a sanitized flask with water from ponds Source to the Ichthyology and Applied Hydrobiology Laboratory of the University of Dschang, Cameroon [14].

Macroscopic examination of Fish

External anatomical examination: A clinical examination was carried out on the live or freshly dead fish. This involved the determination of any clinical signs of disease or abnormalities on the specimens as defined by Noga EJ et al [15]. External clinical signs (body color, scales, presence or absence of hemorrhage, lesions, etc) were observed on the field during a preliminary examination of live fish which were identified and recorded on a data sheet with a code for each fish. Additionally, each abnormality was photographed using a Sony digital camera at 10 mega pixels resolution. This was done in other to avoid inflammatory processes that make diagnosis uncertain or impossible.

Internal anatomical examination: At the laboratory, fishes were dissected by opening each specimen from the anus to the head with a sharp pair of scissors, taking care not to perforate the various organs as intestines. Then, the internal organs (liver and gonads) were removed with forceps and placed in petri dishes. Organs showing clinical sign of disease were placed in a container with 10% formalin for fixation and further histological examination.

Histological examination: The histological technique was carried out on the gonads and liver through the optimized protocol at the laboratory of Mountain University at Bangangté-Cameroon [5].

Parasitological examination of fish: In other to link abnormalities to fish parasites, parasitological examination was done. Hence, Standard parasitological procedures were used for fish examination and identification of ectoparasites and internal helminths respectively [14,16]. An infected fish sample was coded as 1 and uninfected as 0. The prevalence (Pr) or infection rate was calculated according to Bush AO et al [16].

Statistical analysis: The obtained data was entered into Microsoft office Excel 2007 for descriptive statastics and transferred to SPSS for analysis. The chi-square test was used to test significant levels within factors on the prevalence rates and odds-ratios were determined for associated risk factors. Pearson correlation test were used to assess the relationship between the different variables abmalities observed. The significance level (p) considered was set at 5%.

RESULTS

Typology and prevalence of external clinical signs of disease according to fish species

The typology and prevalence of external clinical signs of disease according to fish species are highlighted respectively in Figures 2 and 3. Irrespective of the fish species, a total of 7 abnormalities depending on their anatomical location were observed in 19.5% of the examined fishes. The prevalence of the clinical signs was higher in *Cyprinus carpio* (25.2%) followed by *Oreochromis niloticus* (17.5%) and *Clarias gariepinus* (17.3%). However, no significant difference was observed. Hemorrhage and erosion were about 9 times more frequent ($p<0.05$) than other abnormalities which accounted only for less than 2% each. Hemorrhage, erosion, absence of fin, deformation were common for fishes while red and protruding anus where specific to *Cyprinus carpio* and *Oreochromis niloticus* unlike Exolphtalmia and color alteration that were specific to *Oreochromis niloticus* and *Cyprinus carpio* respectively.

Prevalence of pathologies according to sex of cultured fishes

The prevalence of abnormalities according to the sex of fish is summarized in Table 1. It revealed that female fishes (20.1%) were the most affected compared to males (18.7%). However, no significant effect on the prevalence of abnormalities was recorded ($p>0.05$).

Figure 2: Typology of abnormalities: **Note:** (a) Erosion of the head in *O. niloticus*, (b) Erosion of tail fin in *Clarias gariepinus*; (c) Erosion of tail fin in *C. carpio*; (d) Body in *C. gariepinus* eroded; (e) Deformation of the head and body in *C. gariepinus*; (f) Deformation of the mouth of *C. carpio*.

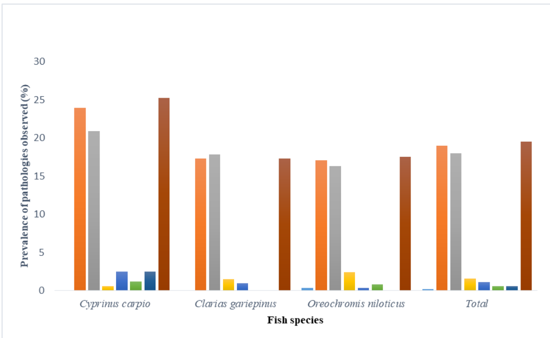

Figure 3: Prevalence of some abnormalities recorded on farmed fish specimens in the West Region of Cameroon. **Note:** (■)-Exopthalmia; (■)-No fin; (■)-Colour alteration; (■)-Hemorrhage; (■)-Deformation; (■)-Anomaly; (■)-Erosion; (■)-Red and protruding anus.

Table 1: Prevalence of abnormalities according to fish sex

Factors	Modalities	N (n)	Prevalence (%)	p-value (X^2)
Sex	Males	998(187)	18.7	0.667 (0.185)
	Females	1256(252)	20.1	

Note: N: Number of fish examined; n: Number of fish with abnormalities.

Typology and prevalence of internal clinical signs of disease related to fish species and organs

Gonad abnormalities: Overall, 2% of collected fishes showed Gonad abnormalities. *Cyprinus carpio* testis was asymmetrically developed and constricted while an ovary of *Clarias gariepinus* was found to be inflamed in the form of an oedema (Figure 4).

Abnormalities in the liver: Liver alterations were recorded in 1.3% of *Clarias gariepinus* collected from fish farms in Menoua division (Figure 5).

Histopathology of fish liver and gonads: The histological sections carried out on the fish livers revealed various tissue damages namely hemorrhagic necrosis while that of ovaries showed hermaphroditism and degeneration of the zona radiate (Figures 6, 7A and 7B).

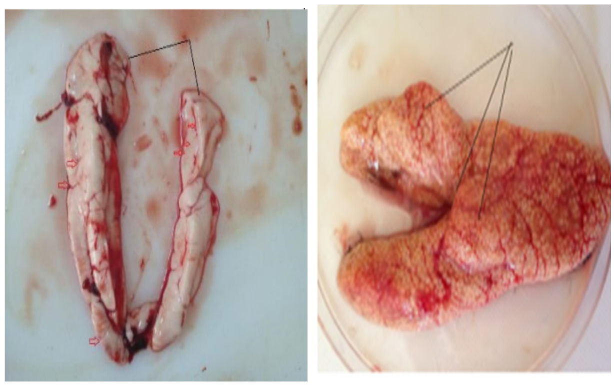

Figure 4: Gonad abnormalities observed in farmed fish from the West Region of Cameroon. **Note:** (A) Testicles with asymmetric development (black dash) and constriction of the gonad (red arrows) in *Cyprinus carpio*; (B) Inflammation of the female gonad in *Clarias gariepinus*.

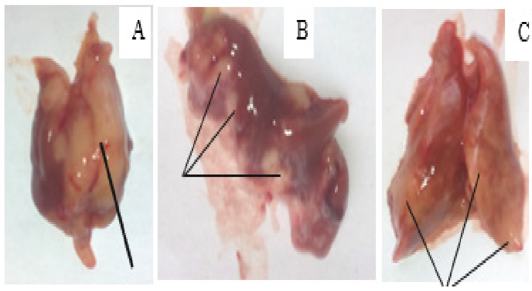

Figure 5: Liver alterations in African catfish. **Note:** (A) Hepatic steatosis (fat black arrow); (B) Nodule inclusion (black arrows) in the liver; (C) Hepatic discoloration (Discoloured areas black arrows).

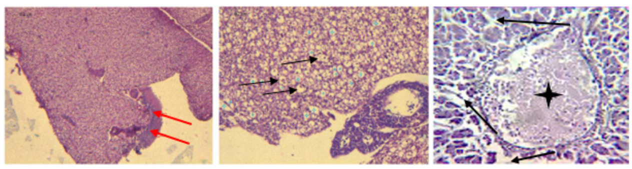

Figure 6: Necrosis and haemorrhage in the fish liver (✦)

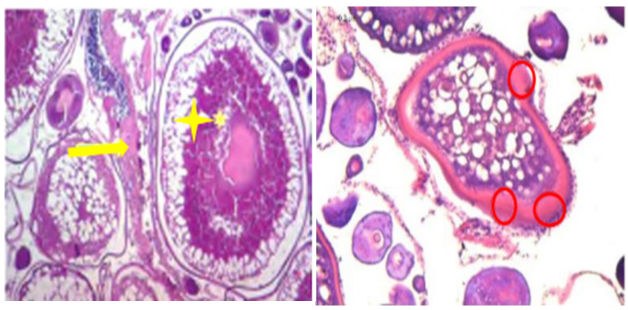

Figure 7: Histopathology of the ovaries. **Note:** (A) Hermaphroditism in *C. gariepinus*: (▬)-Male compartment, (✦)-Atresia; (B) Degeneration of the zona radiata in *O. niloticus*.

Prevalence of ecto and endoparasites: A total of 894 (39.66%) fish were parasitized on the one hand by ectoparasites (34.87%) and on the other hand by endoparasites (8.47%) (Figure 8). 786 fish (34.87%; were infested with ectoparasites consisting of monogeneans (356; 15.79%), protozoa (356; 15.79%) and crustaceans (271; 12.02%). Monogenes consisted of *Gyrodactylus sp*. (285; 12.64%) and *Dactylogyrus sp*. (117; 5.19%). Protozoa consisted of *Myxobolus sp*. (232; 10.29%), *Trichodina sp*. (159; 7.05%) and *Chidonella sp*. (12; 0.53%). The crustaceans were composed of Branchiures comprising *Argulus sp*. (150; 6.65%) and Copepoda (150; 6.65%) including *Lernea sp*. (95; 4.21%) and *Ergasilus sp*. (55; 2.44%). *Gyrodactylus sp*. had the highest parasite infestation rate regardless of fish species while *Chilodonella sp*. had the lowest parasite infestation rate (Figure 8). As for the endoparasites, a total of 191 fish (8.47%) were parasitized by endoparasites including acanthocephalans (109; 4.84%) and nematodes (92; 4.08%). *Acanthocephalus sp*. (109; 4.84%) was the only acanthocephalan identified, while the Nematodes consisted of *Camallanus sp*. (27; 1.20%), *Eustrongylides sp*. (24; 1.06%), *Capillaria sp*. (43; 1.91%) and *Orientatractis sp*. (8; 0.35% [0.18-0.70]). Among these parasites, *Acanthocephalus sp*. recorded the highest prevalence (Figure 8).

Correlation between the prevalence of abnormalities and parasitism: The correlations between the prevalence of abnormalities and parasitism are shown in Table 2. Though all identified parasites except *Chilodonella sp*., *Lernea sp*., *Ergasilus sp*., *Argulus sp*., *Eustrongylides sp* and *Orientatractis sp*. were found on fish with abnormalities, no parasite was significantly (p>0.05) associated with the clinical signs of diseases observed on the host fish.

Correlation between endogenous factors and the occurrence of abnormalities observed in fish: The parasite prevalence was non-significantly (p>0.05) correlated with that of the observed abnormalities (Table 3).

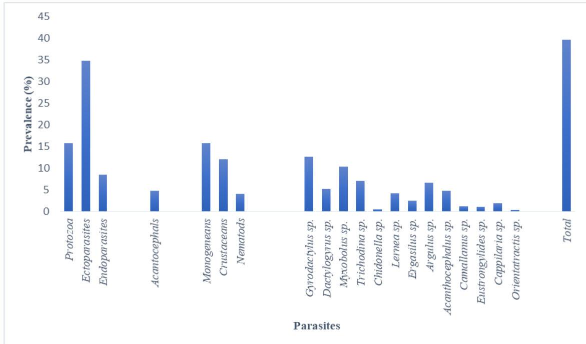

Figure 8: Prevalence of parasites identified in cultured fish in the West Cameroon Region.

Table 2: Correlation between fish abnormalities and parasitism

Parasites	Exolphthalmia (N1 =5)		Hemorrhage (N2=428)		Erosion (N3 =406)		Absence of fin (N4 =36)		Deformation (N5 =25)		Red and protruding anus (N6 =14)		Color alteration (N7=14)		Abnormalities (N=440)	
	n (%)	p	n (%)	P	n (%)	p	n (%)	p	n (%)	p	n (%)	p	n (%)	P	n (%)	p
Monogens																
Gyrodactylus sp.	0(0.0)	1	6(1.3)	0.476	6(1.4)	0.857	1(0.3)	0.207	0(0.0)	0.648	0(0.0)	1	0(0.0)	1	6(1.3)	0.472
Dactylogyrus sp.	0(0.0)	1	21(0.5)	0.332	2(0.5)	0.448	1(0.2)	0.361	0(0.0)	1	0(0.0)	1	0(0.0)	1	2(0.5)	0.328
Protozoa																
Myxobolus sp.	0(0.0)	1	6(1.3)	0.491	7(1.8)	0.571	1(0.3)	0.201	0(0.0)	0.651	1(0.2)	0.295	0(0.0)	1	6(1.3)	0.474
Trichodina sp.	0(0.0)	1	4(1.0)	0.805	4(1.0)	0.802	1(0.2)	0.371	0(0.0)	1	0(0.0)	1	0(0.0)	1	4(1.0)	0.807
Chilodonella sp.	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1
Crustaceans																
Copepods																
Lernea sp.	0(0.0)	1	0(0.0)	0.625	0(0.0)	0.636	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	0.62
Ergasilus sp.	0(0.0)	1	0(0.0)	0.625	0(0.0)	0.636	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	0.62
Branchiura																
Argulus sp.	0(0.0)	1	0(0.0)	0.595	0(0.0)	0.601	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	0.592
Total Ectoparasites	0(0.0)	1	150(3.5)	0.187	16(4)	0.807	1(0.6)	0.253	0(0.0)	0.142	1(0.2)	1	0(0.0)	0.578	15(3.5)	0.151
Acanthocephala																
Acanthocephalus sp.	0(0.0)	0.979	257(0.6)	0.283	2(0.5)	0.713	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	3(0.6)	0.48
Nematods																

Camallanus sp.	0(0.0)	1	86(0.2)	1	1(0.2)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	1(0.2)	1
Eustrongylides sp.	0(0.0)	1	0(0.0)	0.222	1(0.2)	0.699	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	0.223
capillary sp.	0(0.0)	1	5(1.1)	0.16	4(1.0)	0.258	1(0.2)	0.304	1(0.2)	0.224	0(0.0)	1	0(0.0)	1	5(1.1)	0.166
Orientatractis sp.	0(0.0)	1	0(0.0)	0.595	0(0.0)	0.601	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	1	0(0.0)	0.592
Total Endoparasites	0(0.0)	1	8(1.9)	0.341	7(1.8)	0.399	1(0.2)	1	1(0.2)	1	0(0.0)	1	0(0.0)	1	8(1.9)	0.452
Total	0(0.0)	0.703	24(5.5)	0.451	23(5.6)	0.732	1(0.8)	0.172	1(0.2)	0.455	1(0.2)	1	0(0.0)	0.324	24(5.5)	0.74

Note: N: Number of fish examined; n: Number of fish with abnormalities; p: probability of error.

Table 3: Correlation between endogenous factors and the occurrence of abnormalities observed in fish collected from farms in West Cameroon

Risk factors	r	p
Fish species	0.002	0.483
Sex	0.017	0.334
Parasite prevalence	-0.018	0.330

Note: r=correlation coefficients; p: probability of error.

DISCUSSION

The results obtained with regard to abnormalities corroborate those of Samia ASZ et al [7,9,17]. Indeed, exophthalmia has already been reported in fish by several authors. According to, Achat Soraya et Zarouri Samia et al, it was observed in the common carp *Cyprinus carpio* caught in the river at Tala Hamza [7]. Exophthalmia can be of infectious origin or linked to the supersaturation of the medium with gas [18]. Other causes of various origins have also been put forward, namely metabolic disorder, low oxygen content, after exposure of fish to ethanol and nutritional insufficiency (Vitamin A, E deficiency) [19-22]. Hemorrhage in fish is one of the abnormalities that reflect the degradation of the aquatic environment. Its presence is consistent with the descriptions reported by Girard P et al [19]. These authors related this type of abnormality to infectious agents, such as Enterobacteriaceae or viruses and protozoa, trauma, irritation or deficiency in Vitamin C, K [18,19,22,23]. It is also possible that excessive competition between fish will lead to a severe confrontation inducing various hemorrhages.

Deformation of fish is often associated with stunted growth and reduced swimming ability. Generally, deformation in fish is a multifactorial phenomenon and the determination of its origin is not obvious, but heavy metals can cause metabolic alterations that can act on bone metabolism and modify their mineralization [18,24]. But, the time of exposure and the degree of bioaccumulation of contaminants can increase the number of deformed fish [25]. According to Bogé G et al, contamination of the environment by heavy metals can be the cause of a deformation of the vertebral column in fish on the other hand linked this expression to an unsuitable diet and to vitamin deficiencies [26,27]. Moreover, hereditary phenomena are supposed to be at the origin [28]. However, points out that thermal deficits or chemical pollution can cause deformations of this type [29].

Fin erosion has already been reported by different authors for which various causes have been assumed such as fungi and ectoparasites (*Argulose argulus spp*); cadmium; vitamin C deficiency, *Vibrio anguillarum* and predation [19,22,30,31]. Body erosion has also been linked to different sources of disturbance citing infestation by different agents such as bacteria and parasites, nutritional or vitamin deficiencies, unfavorable environmental factors and chemical pollution [19]. They later cause the degradation of the fish's body mucus, which causes the disappearance of this protective layer. The affected organs are subsequently invaded by parasites or fungi, but also by viruses such as carp herpes virus 3 [18]. This is because the skin of fish represents an effective gateway for certain viruses [32]. These organs can also be affected by bacteria like *Aeromonas sorbia* detected in *Garra rufa*, since fish that are in a poor environment due to insufficient water quality such as high levels of nitrites, low levels of dissolved oxygen or high levels of carbon dioxide are more vulnerable to [33]. Erosions and deformations can be linked to different physical factors such as: gas oversaturation, hypoxia, too low temperature, salinity, radioactivity, electric shocks, organochlorine compounds (pesticides, herbicides), heavy metals (Cd, Pb), parasites, bacteria, viruses or trauma (capture, predation). It can also be related to nutritional deficiencies, especially in vitamins [19], such as vitamins (A, C and D) [34]. It was also showed the separation of the fins was due to the exposure of fish to fertilizers (potash and ammonium salts) [30].

The change in phenotype (color alteration) during an established life stage was associated to the response to environmental interactions, but also with the transition between two stages of development phenotypically pre-adaptive to their ancestral ecosystems [20]. Color alterations in fish can be linked to several factors. Indeed, it can be of physiological origins, namely stress related to capture, but also a physical origin including, among others, hypoxia, excess CO_2, gas oversaturation, insufficient mineralization of the water. It can also be of accidental origin (haemorrhage, trauma, irritation, blindness), genetic (hereditary diseases) or nutritional (vitamin deficiencies). Finally, a change in color can also be due to bacterial, parasitic (Myxosporidia, microsporidia) and viral infections [30,19].

It appears from this study that the abnormalities identified would have a negative impact on the overweight of its host, probably by disturbing its physiology. The abnormalities identified, although not significantly associated with the identified parasites, can be detrimental to fish production. Indeed, these superficial and/or deep lesions constitute entry points for secondary infections [35]. They can create serious dysfunctions for the individual which can lead to a drop in fertility [36]. Indeed, the clinical signs of naturally infected fish revealed respiratory distress, swimming at the surface and slow movements. These signs can be attributed to massive mucous secretions in the gills which can be used to dilute the irritation and act as a defense mechanism against infestation [37]. These results are almost similar to other reports that highlighted the abnormalities associated with the clinical signs mentioned above [36,38,39]. It would therefore be essential to monitor their appearance in fish farms in order to avoid such damage in fish farming.

Histopathological changes in the skin and gills of infested fish were recorded and the results are similar to those recorded by Aly HA et al [40]. Indeed, skin and gill lesions can be induced by feeding activity, attachment and locomotion of monogeneans causing massive destruction of respiratory epithelial cells and/or skin cells as well as ciliated trichodins on the skin. These results agree with the work of Gado MS et al [41,42]. In the gills, hyperplasia occurs takes place between the gill lamellae and the parasites then feed on the newly produced cells and damage the gill tissues. Thus, the branchial epithelium is completely destroyed, leaving large denuded areas between the filaments. Perforated basement membrane and capillaries cause bleeding. This condition disrupts breathing [42]. Eventually, the fish die of suffocation [42]. The physical presence of *Trichodina sp*. and *Myxobolus sp* localized in the vessels damages the gills and opens the gateway for secondary infection. This leads to high mortality in the nursery operating system in carp [43]. The gills were frayed, congested and slimy with a mottled appearance. The mottled appearance can be attributed to the destruction of efferent vessels. This leads to rapid occlusions of the vessel and then a thrombus forms resulting in ischemia, which in turn leads to necrosis [36,41]. Other cases showed pale gills with destruction of gill filaments. These results are consistent with those reported by Eissa AE et al [44,45]. Additionally, Vinoth R et al, found that infested fish had extremely pale gills, indicating that the gills were seriously affected [46]. Histopathological findings from the affected intestines showed massive destruction, necrosis, atrophy as well as leukocyte infiltration. These results are in agreement with the work of [45,47]. These results can be attributed to the parasite-induced mechanical injury and its feeding on the delicate tissues on the circulating blood resulting in hemorrhage. Slow, rapid blood clot, thrombus, ischemia, finally necrosis and widespread damage to the gastrointestinal tract [48-50]. In view of the impact of parasites at the tissue level, identifying fish diseases and their causative agents is necessary to initiate preventive measures against pathogens. It would therefore be essential to monitor the appearance of parasites in fish farms given the growing involvement of fish farming on a daily basis.

CONCLUSION

Several abnormalites have been recorded on the external and internal anatomy of the fish. However, they were not significantly associated with the fish species, their sex and the prevalence of parasites. The histological sections performed showed various tissue and cellular alterations in the affected organs. It will be advisable to extend these studies to other agroecological zones of Cameroon with a view to establishing a map of the biosecurity measures, prevalence of recurrent pathologies in fish farms according to the seasons, and the means of prevention and control of these pathologies. This will contribute effectively to the increase of national fish farming production. Also, a microbiological study of the different organs of the affected fish, and a microbiological analysis of the water should be carried out.

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CONFLICT OF INTEREST

There is no conflict of interest.

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