

Parasitic and non-parasitic conditions affecting farmed and wild cichlids in Tanzania

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SUMMARY

Infectious fish diseases are among the known contributing factor in reduced productivity of fish farming enterprises. Despite of the growing importance of global fish farming industry, research in fish and other aquatic stocks relevant to Tanzania is limited. This paper presents preliminary results of the ongoing investigation on fish mortalities which occurred in fish farm located in Kibaha District. The paper also present preliminary results of formalin fixed samples received from other parts of Tanzania. In all the samples; branchitis, gill deformity, and intracellular chlamydia like organisms were the major findings regardless of the source. Other findings include encysted trematode metacercaria in different anatomical locations accompanied with variable pathomorphological changes to the host tissues. Interestingly, mortalities ceased in the affected farm after replenishment of water supply suggesting that either poor water quality was the main predisposing factor or aggravated the observed disease conditions. Therefore maintenance of water quality and or water replacement is recommended as the first intervention measure where poor water quality is strongly suspected to be associated with mortalities in fish farms. Further studies on the pathobiological characteristic of the observed infectious organisms will provide more insights on the suspected relationships between the environmental factors in one hand and progression of the observed pathological changes in fish.

Key words: Metacercaria, Fish, Chlamydia, Tilapia, Pathology, Lake Victoria

INTRODUCTION

Global demand for protein rich food remains on the rise making the insufficiently available sources to be beyond the reach of the poor due to high market prices. It is estimated that in order to maintain the current per capita consumption level of protein, world aquaculture production will need to be increased up to 80 million tonnes in the next 30 years (FAO, 2006). The rising global demand for fish and declining production from traditional sources of fish has created positive incentive for growth of aquaculture farming in Tanzania. In the last two decades alone aquaculture fish production increased from almost zero in early 2000 up to the estimated 5000 metric tons by the year 2016 (FAO, 2007; MLFD, 2016). Nonetheless, aquaculture fish farming in Tanzania is still lagging behind compared to other regional neighbours such as Kenya and Uganda with estimated annual production level of approximately 20,000 and 100,000 metric tons respectively by the year

2016 (FAO, 2007). Among the challenges facing fisheries sector in Tanzania is inadequate research and support in tackling health challenges related to aquatic health resources. In order to support the current steady growth in fish farming, a collective and multisector effort is needed including strengthening research and dissemination of results to the end users for the improvement of fish farming, processing and marketing practices. This study presents preliminary results of the ongoing investigation of recently reported cases of fish mortalities under intensive fish farming systems with the aim of finding the cause and design mitigation measures. In addition, results on parasitic ecology of wild and farmed fish as well as their significance in fish health are also discussed.

MATERIALS AND METHODS

Fish samples suspected of being poisoned with ammonia and were reared in collapsible and movable plastic fish rearing tanks using

recirculation aquaculture system. Water quality analysis was done along with post-mortem examination of dead fish. Samples for histopathology were collected and preserved in 10% neutral buffered formalin fixative until analysis. Swabs were also collected and sent for bacteriology and mycology screening. Fish samples without history illness from different sources and that were collected during routine diagnostic training for undergraduate and graduate students were also received for histopathological analysis. All tissues intended for histopathological analysis remained in 10% neutral buffered formalin for at least 24 hours before further processing, sectioning and staining. Hematoxylin and eosin (H&E) stained sections were examined using bright field Olympus microscope (BH2, Olympus) mounted with digital camera (Moticam Pro 285A, Motic Microscopy). Descriptive dot plot was generated using open source R software (Team, 2013) and ggplot2 R-package (Wickham, 2009)

RESULTS

Ammonia toxicity in recirculation aquaculture system

The farm had a total of 6 collapsible and movable plastic fish rearing tanks using recirculation aquaculture system (RAS) and each tank contained 2500 fish aged between two and four months. Mortalities were observed in one of the tank with fish aged three months and a total of 350 deaths were recorded. Measured water pH level was 8.0 and 8.2 in the affected tank and bio filter respectively. Water ammonia level was 2 mg/L in the tank and 0.2mg/L in the biofilter. The nitrite and nitrate amount in the affected tank were 0mg/L each. Interestingly, changing from RAS to direct water supply stopped mortality. Based on the measured parameters and day water temperature of 28 degrees of Celsius, the toxic non-ionised form of ammonia was estimated to reach 0.13mg/L based on the formula described by (Emerson *et al.*, 1975). Clinically, some fish had excessive mucus and red/bleeding gills with some gasping for air on water surface.

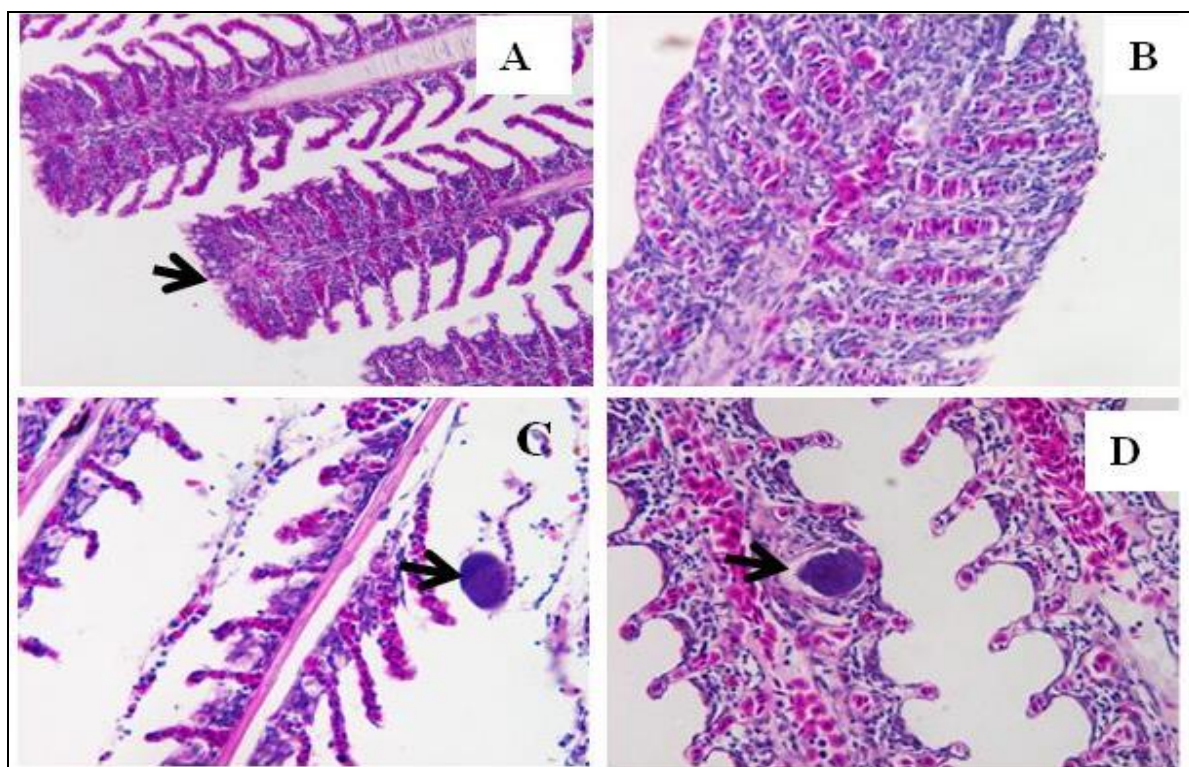


Figure 1. Fish sample suspected of having ammonia poisoning. Branchitis with lamellar hyperplasia, lamella fusion and hydropic degeneration (arrow) at the apex (A), severe lamella hyperplasia and fusion (B), basophilic cytoplasmic inclusions (arrow) and lamellar hyperplasia at the junction of secondary lamella (C-D). Images taken from Hematoxylin and eosin (H&E) stained gill tissue sections. Image magnification (A: 40x, B-C: 100x, D: 200x).

Laboratory examination of formalin fixed tissues of fish revealed lamella hyperplasia accompanied with fusion of secondary lamella, hydropic degeneration and telangiectasia at the tips of secondary lamella. Notably lamella hyperplasia and fusion was more severe at the apex and severity decreased towards the base of primary lamella. Occasionally, enlarged epithelial cells with pale to basophilic cytoplasmic inclusions were observed in the fish lamellae (Figure 1).

Incidental findings during post-mortem fish examination

A total of 72 fish of different sizes (Figure 2) collected from the shores of Lake Victoria and measured water quality parameters indicated slightly acidic pH (Table 1). Closer examination of the fish revealed white spots on the gill surfaces (not shown). In addition, 10 fish from aquaculture farm were examined as part of routine undergraduate students training. Fish were described as normal in their natural environment and did not demonstrate any sign of ill-health but some fish showed presence of white spots on the gills or black spots in different locations including skin, fins, eyes and muscular tissues.

Table 1. Measured water quality parameters at different sampling points along Lake Victoria

Sampling points	Turbidity (NTU)	Salinity	pH	Water temperature
A	2.24	0	4.2	26
B	2.12	0	5.07	25
C	2.85	0	5.14	25

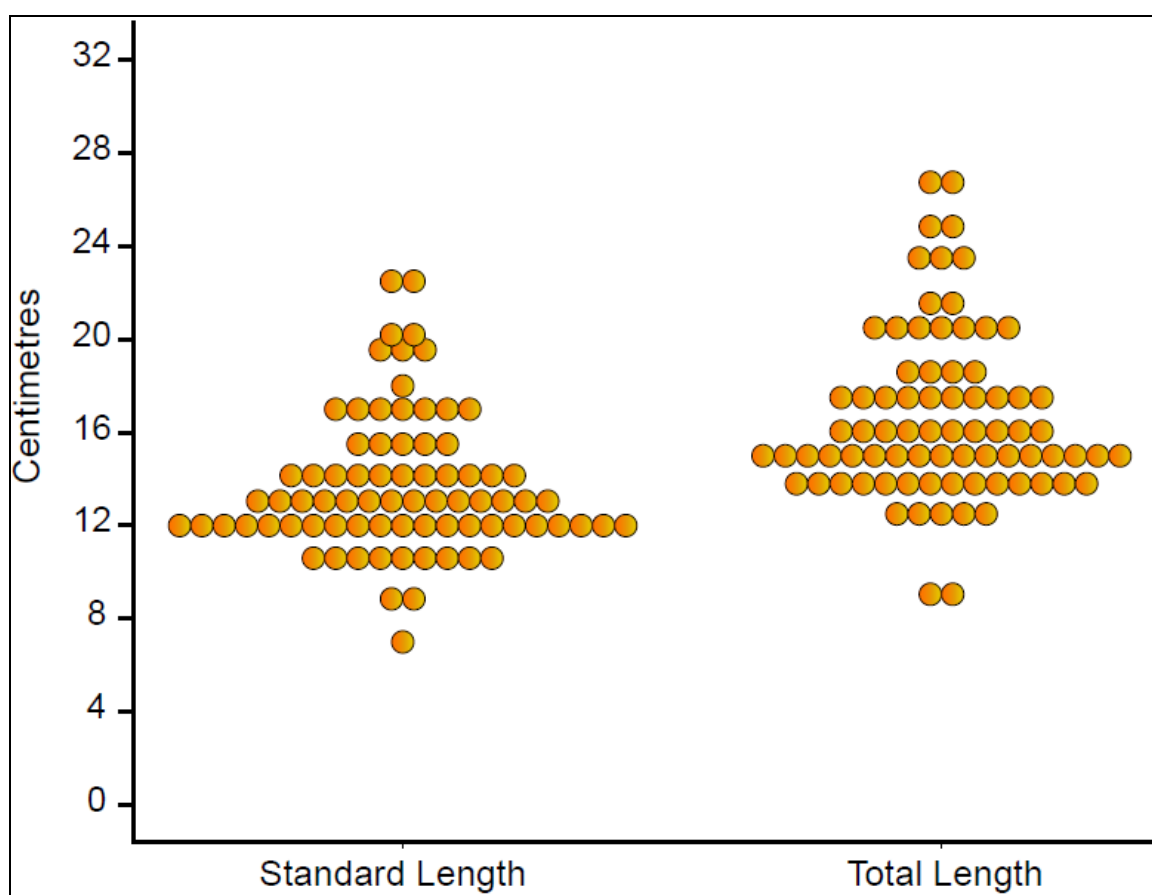


Figure 2. Dot plots of measured fish sizes (centimetres), n=72, each dot represent one fish.

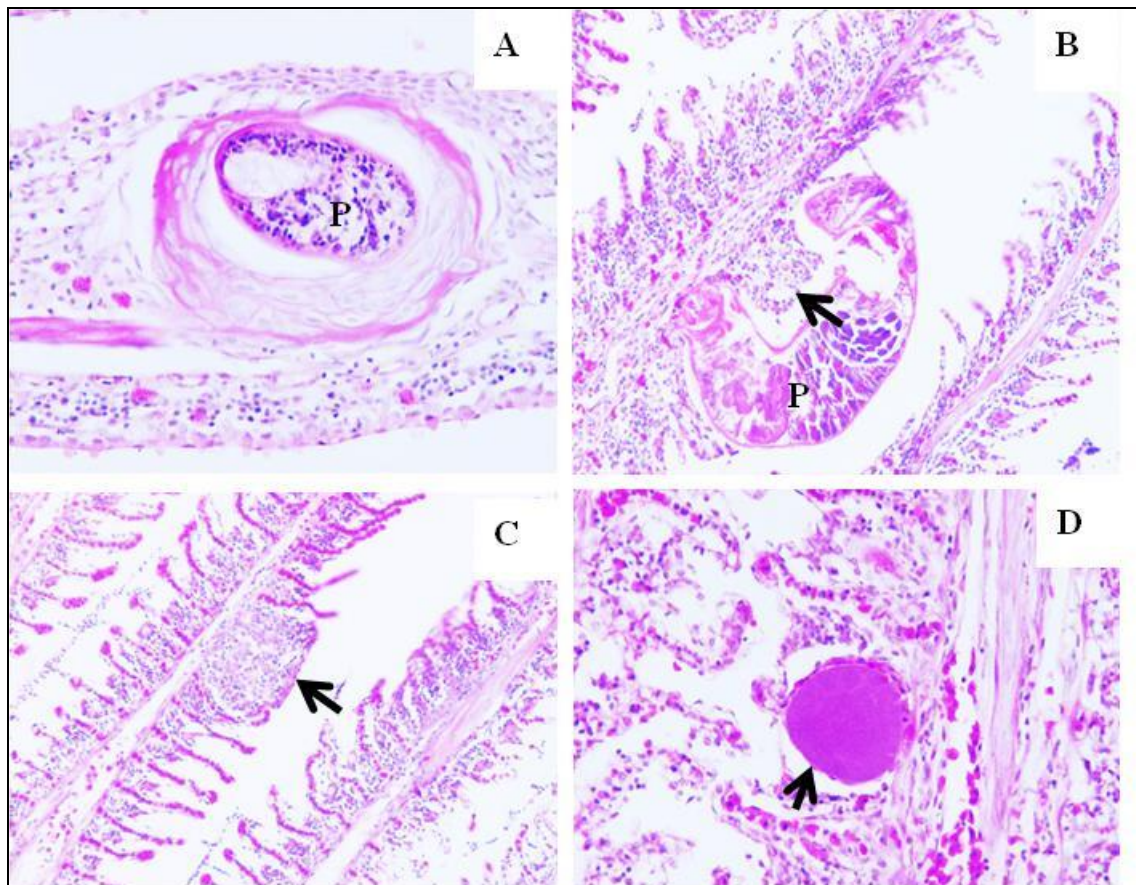


Figure 3. Fish sample from Lake Victoria, Nyegezi area. Parasitic metacercaria (P) in bony cartilage (A), Parasitic (P) attachment between two primary lamella (P) causing lamellar hyperplasia and fusion (arrow)(B), Telangiectasia at the tip of lamella and nodular lamella fusion (arrow)(C), basophilic cytoplasmic inclusions (arrow) (D). Images taken from Hematoxylin and eosin (H&E) stained gill tissue sections. Image magnification (A-B: 100x, C: 40x), and D: 200x).

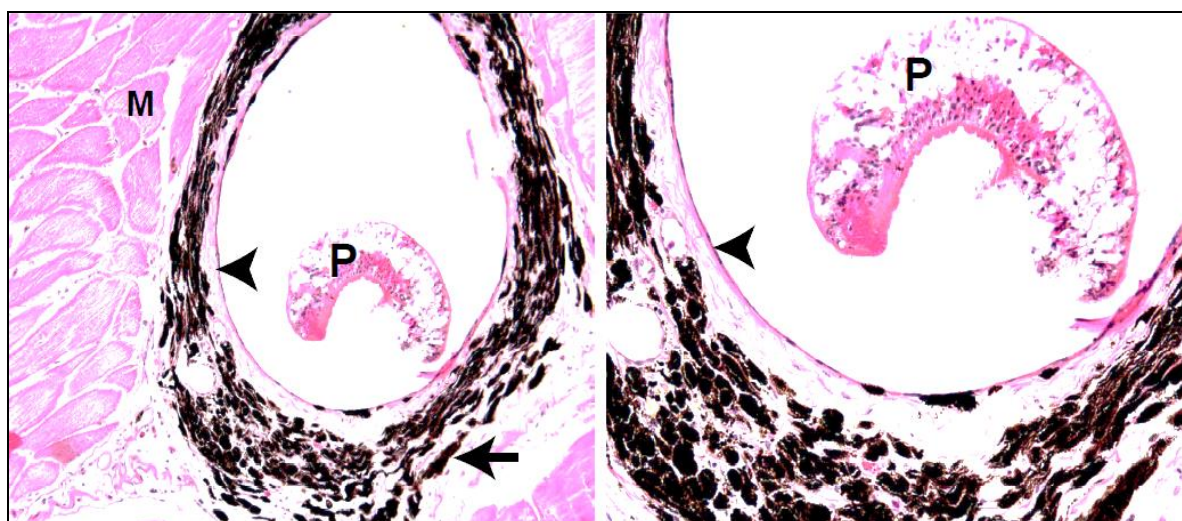


Figure 4. Samples from farmed Tilapia in Songea, southern Tanzania showing *Neascus* larvae (P) in fish fillet (M) surrounded by cyst wall (arrow) and black pigment

The affected areas were sampled and submitted to the laboratory for further investigations. Histopathological examination identified 11 out of 72 fish with trematode larvae encysted in the gill tissues. In addition to the parasitic larvae, three fish were also infected with intracellular basophilic cytoplasmic inclusions (Figure 3), and two fish were infected with un-identified protozoaone in the muscles and one in the gills respectively (data not shown). Morphologically, all infected gills showed gill hyperplasia and one fish showed a nodular hyperplastic lesion formed due to localized lamellar fusion (Figure 3). All 10 samples from cultured fish were affected with *Neascus spp* and showed gill hyperplasia in addition to accumulation of black pigments (data not shown). Affected muscular tissues did not show morphological alterations apart from cyst formation and accumulation of black pigments (Figure 4).

DISCUSSION

The importance of water quality and water quality maintenance in fish health is well documented (Stavrescu-Bedivan *et al.*, 2016). For example, high ammonia levels coupled with high temperature and pH can become lethal due to an increase in un-ionised form of ammonia (Stone *et al.*, 2013). The level of non-ionised form of ammonia which is toxic to fish depends on temperature, pH and total measured ammonia levels in water (Emerson *et al.*, 1975). In this study, non-ionised form of ammonia was estimated to be higher than the recommended limit of 0.05mg/L for most fish species (Emerson *et al.*, 1975). Indeed, severe pathological changes in the fish respiratory system and high fish mortality were observed consistent with ammonia toxicity and pathological changes reported elsewhere (Yoon *et al.*, 2015). This is similar to what was described by Loh (2014) that, Gills from ammonia-treated fish displays severe histological and ultrastructural alterations, including hyperplasia, hypertrophy, fusion and aneurysms of secondary lamellae. Although some of the pathological changes observed such as apical lamella fusion can also occur in other conditions such as pantothenic acid deficiency (Olsvik *et al.*, 2013), the fact that fish mortality ceased by merely water replenishment suggest that mortalities were not related to nutritional deficiency. Furthermore, the presence of mild form of epitheliocystis characterized by occasional intracellular basophilic inclusions was

less likely to induce large scale fish mortality (Figure 3). Although severe infection can cause spontaneous mortalities under intensive fish rearing systems and poor water quality (unpublished report). Furthermore, fish can survive mild epitheliocystis without causing ill-effects under favourable water conditions (Figure 3; Nowak *et al.*, 2006).

In addition to ammonia toxicity, trematode larvae and epitheliocystis were also found in fish along the shores of Lake Victoria. The presence of trematode fish parasites in Lake Victoria cichlids has been reported previously (Akoll *et al.*, 2012; Paperna, 1980). However, the effect of these parasites on fish health in natural water bodies is not clearly understood. Patho-morphological presentation of trematode related lesions vary depending on location in the fish body as well as the species of parasites. Some parasites such as *Centrocestus spp* are known to be frequently found in bony cartilages of gills whereas other parasites such as *Neascus spp* can form cysts in any parts of the body (Paperna, 1980). Lesions caused by *Neascus spp* can be distinguished from others due to the formation of black spots on location where the larvae are encysted as a result of pigment mobilization (Figure 4). Although parasites of the genus *Neascus* has been reported in the northern parts of the lake in Ugandan side (Paperna, 1980), this species and its associated lesions were not detected in all 72 fish collected in Nyegezi area in Mwanza Tanzania. The reasons of not detecting this parasite could be its absence in the area or unfavourable environmental factors as it has been described previously that the infectivity of parasites can vary with water temperature (Goedknegt *et al.*, 2015). However, this parasite was identified in fish samples collected from southern highlands of Tanzania where average water temperature is relatively cooler. Based on morphology and site of cysts formation, majority of the parasites can be grouped as *Neascus*, *Dactylogyrus* and *Centrocestus spp* respectively pending definitive identification (Figure 3 and 4). Despite of harbouring these parasites at various tissues, there were no noticeable ill-health effects on the affected fish probably because the fish have adapted to the presence of parasites. The presence of epitheliocystis in wild cichlids and other fish species has been reported in different countries with variable consequences (Steigen *et al.*, 2018). This disease is known to be caused by an obligate intracellular chlamydia like

organisms (Nowak *et al.*, 2006). The severity of this disease is usually high under intensive system compared to natural water bodies (Nowak *et al.*, 2006). It is therefore not uncommon for the examined fish from extensive environment of Lake Victoria to show no signs of ill-health related to epitheliocystis

Lastly, the recorded pH along the shores of Lake Victoria was relatively low (Table 1). The cause of low pH was not immediately established; however the pH is likely to be influenced by high density of organic matter along the shores where the measurement was taken. Despite of the water pH being relatively low, it is unlikely that such level of pH can have effects on fish health due to the possibility of free movement within lake compared to intensive aquaculture systems. However, further studies on the pathobiological characteristic of the observed infectious organisms and environmental factors are required to provide a more understanding on the pathogenicity of the observed organisms in fish.

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