

Full Length Research Paper

Histopathological changes induced by copepoda parasites infections on the gills of economically important fish mugilidae (*Liza falcipinnis* and *Mugil cephalus*) from Ganvie area of Lac Nokoue, Republic of Benin

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Histopathological changes induced by copepods parasitic infection on the gills of economically important fish, *Mugil cephalus* and *Liza falcipinnis* from Ganvie area of Lac Nokoue were examined from December 2011 to July 2012. Histopathological changes shows that the nature of damage observed in the gill of both *M. cephalus* and *L. falcipinnis* remained the same. Histopathological observation reveals serious damage of lamellae and gill filaments due to attachment and feeding of copepods. The resultant hypertrophy of the underlying epithelial reducing the surface area for effective respiration, could lead to suffocation, particularly at high temperature. The histopathological changes enacted by the copepods parasites will eventually lead to reduced growth, low productivity and mortality resulting in economic loss.

Key words: Copepoda, parasite, histopathology, Mugilidae, Ganvie.

INTRODUCTION

Parasites have recently been highlighted as serious pathogenic problems in mullet fish in marine and brackish water. Among the parasites, copepod family is commonly found on fishes cultured in brackish water (Noor et al., 2012). With the developments of brackish and marine aquaculture, the importance of parasitic copepods as disease causing agents has become more obvious. Copepods parasites attached to gill filaments produce these small foci of eroded host tissue. Apparently, feeding involves external digestion (Halisch, 1940; Kabata, 1970); parasites produce digestive secretions

which partially dissolve tissue, allowing easier ingestion. As the number of attached parasites increases, the destruction of respiratory epithelium progresses. Erosion can extend beyond the epithelial lining, resulting in obstructed branchial blood vessels. Irritation often results in responsive hyperplasia of epithelium, which as infestation intensifies, extends over considerable areas (Kabata, 1970; Paperna Overstreet, 1981). Both processes reduce the respiratory function of the gills.

Parasitic surveys of copepods (Figure 1) have been described from different hosts and locations in different

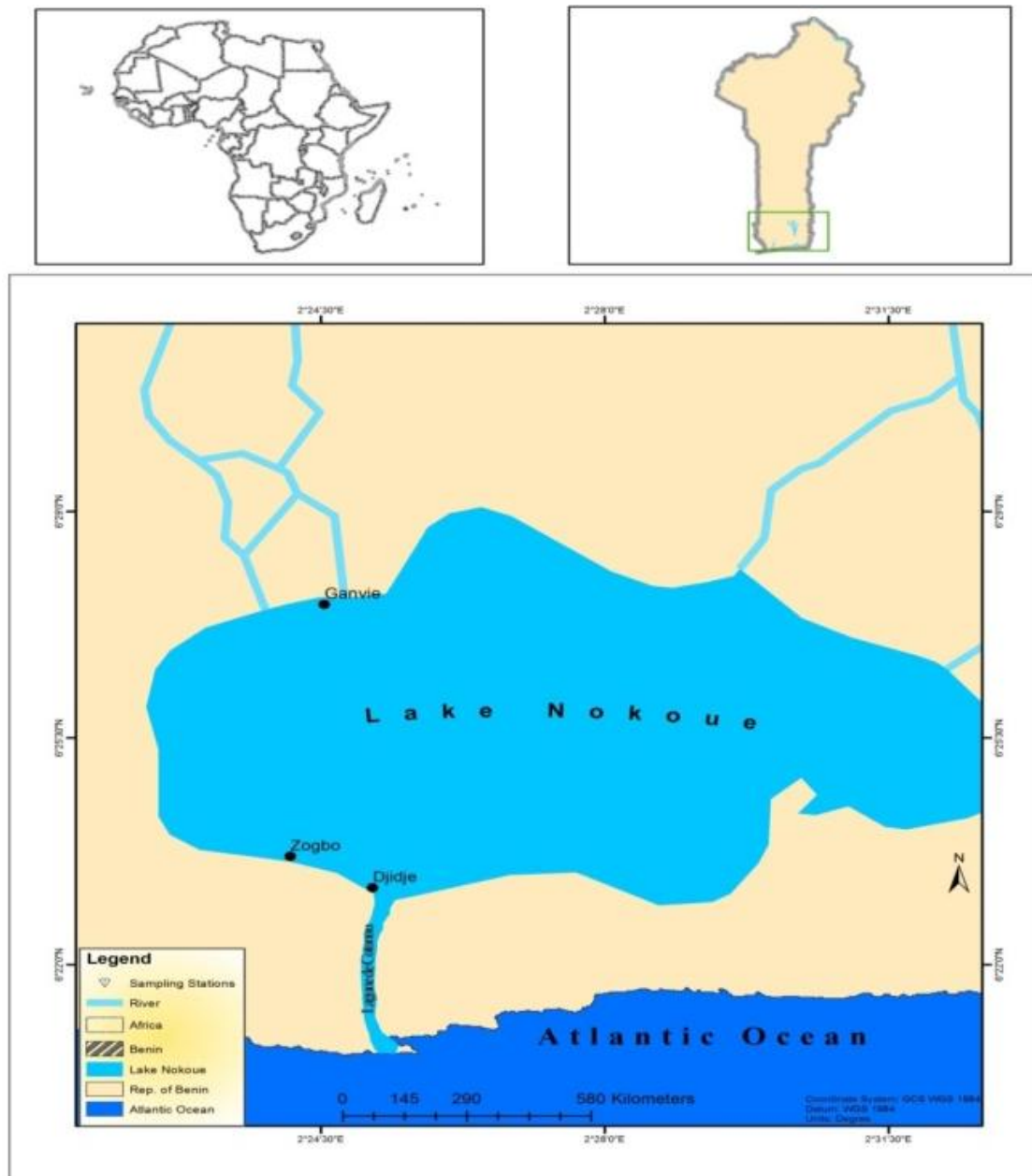


Figure 1. Location of Ganvie in lake Nokoue lagoon.

parts of the world: In Israel, Parpena and Lahav (1971), in Turkey, Koyun et al (2007), in Mexico, Suárez-Morales and Santana-Piñeros (2008), in Canada (Hogans,1989), in Sri Lanka, Vinobaba (2007), in Nigeria, Aladetohun et al. (2013) and in Republic of Benin, Aladetohun et al. (2013). However, histopathological impact of the parasites on their hosts remains to be determined. The aim of this study was to determine the host-parasite interaction, with specific reference to the pathology induced by copepod parasites on economically important fish *Mugil cephalus* and *Liza falcipinnis* in the Ganvie area of Lac Nokoue lagoon in Republic of Benin.

MATERIALS AND METHODS

The study area

The study site, Ganvie area (Figure 1) is located at the northern part of the lake Nokoue lagoon, near the floating village of Ganvie where the water is characterized by a high level of organic pollution (Laleye et al., 2003).

Lake Nokoue (Figure 1) is the largest lagoon (Moreau, 2004), it is a shallow, sub-tropical coastal lagoon ($6^{\circ}25'N$, $2^{\circ}36'E$) with surface of 150 km and stretches 20 km in its east-west direction by 11 km in the north-south direction (Laleye et al., 2003). Lake Nokoue opens directly into the Atlantic Ocean through channel at Cotonou which is about 24.5 km long.

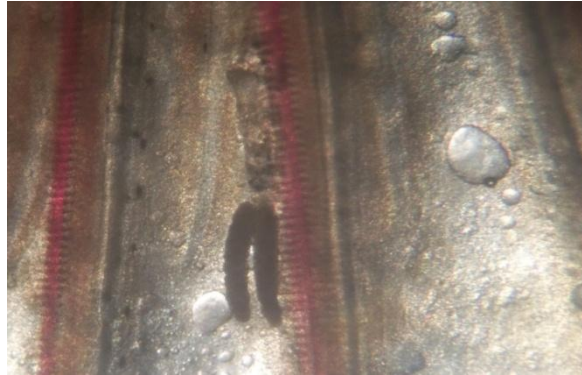


Figure 2. Attachment of copepod in the gill of host fish.

Table 1. Prevalence and mean intensity of *Ergasilus latus* infections in *M. cephalus* and *L. falcipinnis* from Ganvie area of Lac Nokoue.

Species of fish	No of fish examined	No of fish infested	No of copapod parasite	Percentage prevalence (%) for MC and LF	Mean intensity for MC and LF
<i>M. cephalus</i>	115	90	18		
<i>L. falcipinnis</i>	132	110	15	80.97	0.165
Total	247	200	33		

Histopathological analysis

Damaged fish tissues (gills) (Figure 2) were taken from the parasite attachment area of infested fishes and were cut out in fresh condition fixed in 10% buffered neutral formalin (Buoins fluid). The tissues were then processed using a 24 h automatic tissue processor for a time ranging from 17 to 19 h to process. The tissue processor contains 12 beakers, 10 glass beakers and 3 thermostatically controlled electric metal beakers containing paraffin wax.

The tissue (gills) were transferred to Beaker 1, containing 10% formal saline for complete fixation and then transferred to beaker 2-8 containing different ascending grades of dehydrating fluids (alcohol ranging from 70% alcohol to absolute alcohol (isopropyl alcohol, which helps in removing water from the tissue samples and then into beakers 9 and 10 containing clearing agents[xylene I and II] which completely clears the dehydrating agent off the tissue sample.

The tissue samples were later transferred into beakers 11 and 12 containing embedding agent, that is, molten paraffin wax which provides solid support upon embedding.

The tissues after being processed are embedded using an automatic embedding centre. Embedding is a process of submerging a tissue in a metal plastic disposable embedding mould containing molten paraffin wax, which became solidified when it was cold. This formed a support medium for the tissue during sectioning.

Sections of the tissue were cut using a microtome and were placed in a clean grease free slide which was then placed on a hot plate for 30 min in order for section to adhere to the slides. The staining method used was the H&E staining method. This method was used in order to demonstrate the general structure of the tissues. These were then dewaxed in xylene. The processed section were later taken to water by using descending grades of alcohol, that is, from absolute alcohol >95% alcohol, >70% alcohol,

water. It was stained in haematoxylin for 10 min, then rinsed in water.

These were differentiated in 1% acid alcohol (a dip), rinsed in water and then blued in tap water for 5 min. This was counter stained in 1% Eosin for 2-5 min and rinsed in tap water, then dehydrated using ascending grades of alcohol (70% alcohol, 95% alcohol absolute). These were cleared in xylene, mounted using D.P.X (a mountant) and viewed under the microscope.

RESULTS

M. cephalus and *L. falcipinnis* were found to be infested ergasilids (*Ergasilus latus*, *Ergasilus lizae* and *Nipergasilus bora*), attached to the gill filament (Figure 2). The list of hosts sites species with corresponding hosts parasites species are given in the Tables 1, 2, 3 and 4. The prevalence and intensity of infection varied with the host fish (Tables 1, 2, 3 and 4).

Adult females ergasilids are attached to primary lamella (gill fillaments) of the host fish with their claw-like secondary antennae close to the gill arch near the base of the fillament (Figure 2). The lamellae cells were pyknotic and degenerating as a result of second antennae insertions by the ergasili parasites (Figures 3 and 4). The infested gill rakers and gill lamellae were almost totally lost (Figures 3 and 4). It was also observed that the proliferated epithelium contained hypertrophic cells with intercellular spaces in which wandering cells such as blood granulocytes, lymphocytes, vacuole cells and coarse eosinophilic granular cells were evident.

Table 2. Prevalence and mean intensity of *Ergasilus lizae* infections in *M. cephalus* and *L. falcipinnis* from Ganvie area of Lac Nokoue.

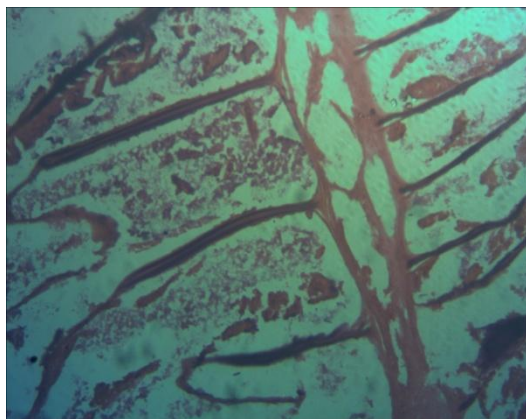
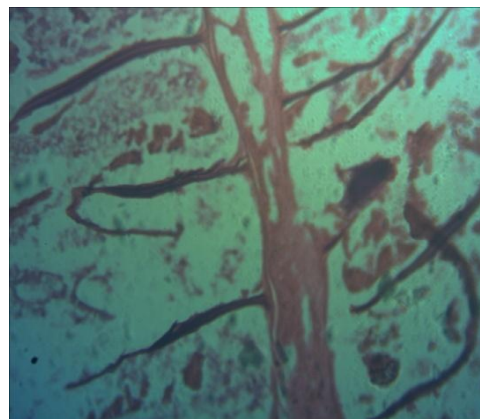
Species of fish	No of fish examined	No of fish infested	No of copapod	Percentage prevalence (%) for MC and LF	Mean intensity for MC and LF
<i>M. cephalus</i>	115	90	20	80.97	0.135
<i>L. falcipinnis</i>	132	110	7		
Total	247	200	27		

Table 3. Prevalence and mean intensity of *Nipergasilus bora* infections in *M. cephalus* and *L. falcipinnis* from Ganvie area of Lac Nokoue.

Species of fish	No of fish examined	No of fish infested	No of copepod	Percentage prevalence (%) for MC and LF	Mean intensity for MC and LF
<i>M. cephalus</i>	115	90	56	80.97	0.77
<i>L. falcipinnis</i>	132	110	98		
Total	247	200	154		

Table 4. Summary of the percentage prevalence and mean intensity of *E. latus*, *E. lizae* and *N. borapar* parasite in *M. cephalus* and *L. falcipinnis* from Ganvie area of Lac Nokoue.

Parasite	No of fish examined		Total	No of infested fish		Total	Percentage prevalence (%)	No of copepod parasite		Total	Mean intensity
	<i>M. cephalus</i>	<i>L. falcipinnis</i>		M C	L F			M C	L F		
<i>E. latus</i>	115	132	247	90	110	200	80.97	18	15	33	0.165
<i>E. lizae</i>	115	132	247	90	110	200	80.97	20	7	27	0.135
<i>N. bora</i>	115	132	247	90	110	200	80.97	56	98	154	0.77

**Figure 3.** High destruction of gill filaments, gill lamella, gill rakers, gill arches and detachment of epithelial cells in lamella of *M. cephalus*.**Figure 4.** High destruction of gill filaments, gill lamella, gill rakers, gill arches and detachment of epithelial cells in Lamella of *L. falcipinnis*.

DISCUSSION

Copepods feed by browsing on the fish gill epithelium or by ingesting blood from ruptured blood vessels.

Apparently, feeding involves external digestion (Halisch, 1940; Kabata, 1970); parasites produce digestive secretions which partially dissolve tissue, allowing easy

ingestion.

The research carried out shows the gill beneath the parasites, it seems like a cavity which is probably related to the enzymatic reaction of the gill epithelium beneath the parasite. This report coincides with that of Vinobaba (2007) in his work on histopathological changes induced by ergasilid copepod infections on the gills of food fish from Batticaloa lagoon, Sri Lanka.

In this work, the copepod parasites found caused changes on the gill fillaments through feeding. Both the gill lamella and the gill arch and gills rakers were badly damaged. This was also reported by Rameshkumar and Samuthirapandian (2013) on histopathological changes in the skins and gills of some marine fishes, Overstreet (1978), Ben-Hassin (1983), Morella and Garippa (2001) in his work on parasites of grey mullets from Mistras lagoon, Western Meditar-ranean, Ben-Hassin (1983) in his work on copepod parasites of mugilidae.

This research work reveals lost of gill fillament and totally almost missing. Parperna and Lahav (1971), reported on copepod parasite (*Ergasilus lizae*) in grey mullets in Israel and Vinobaba (2007) in his work on histopathological changes induced by ergasilid copepod infections on the gills of food fish from Batticaloa lagoon also gave the same report.

Copepod parasites attaches to the host using various appendages modified for gasping and this activity can led to secondary infection by pathogenic organism (e.g bacteria, fungi, and virus) and cause mass mortality.

High intensity of infection of these copepods may lead to serious damage of the gills and therefore show pronounced impact on the histology and lead to mortality (Vinobaba, 2007). Environmental change, especially habitat degradation by anthropogenic pollutants and oceanographic alterations induced by climatic changes and temperature dependent can influence parasitic-host interaction (Abalaka et al., 2010).

In conclusion, the resultant hypertrophy of the underlying epithelial reducing the surface area for effective respiration could lead to suffocation, particularly at high temperature. The histopathological changes enacted by the copepods parasites will eventually lead to reduced growth, low productivity and mortality resulting in economic loss.

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