

Effects of Water Quality and Monogenean Parasite in the Gills of Freshwater Cat Fish, *Hemibagrus nemurus* Valenciennes 1840

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Abstract: The Study on *Hemibagrus nemurus* (Valenciennes, 1840) gills was designed to investigate the influence of monogenean irritation coupled with water quality condition of the pond (earthen pond) in Perlak, Pahang. Three hundred and eighty fish host were examined, their gills were excised and fixed in 3% glutaraldehyde for scanning electron microscopy and in bouin's solution for histology. In the infected fish gills, the pathological alterations observed such as proliferative, degenerative and necrotic changes in the epithelium of gill filaments. In the secondary lamellae, telangiectasia, fusion of secondary lamellae and excessive mucous cells proliferation were observed. Correlation was made between water quality parameters and intensity of monogenean infestation in the ponds. It can be concluded that gill alterations as a result of monogeneans irritation and poor water quality in fish ponds may serve as a sensitive biomarker for pollutants.

Key words: Gills, *Hemibagrus nemurus*, monogenean, pathological alteration, water quality

INTRODUCTION

Fish gill is very sensitive to physical and chemical alterations of the aquatic medium and to any change in the composition of the environment which is an important indicator of water borne toxicants (Ogundiran *et al.*, 2009). Fish gill also play a vital role, as they are the main sites of gaseous exchanges, which involved osmoregulation, acid-base balance, excretion of nitrogenous compounds (Pereira and Caetano, 2009) and harbors many microbes. This is because they are directly in contact with the aquatic habitat. From a morphological and physiological point of view, the gill is very complex organ, involved not only in gas exchange, ion exchange and acid-base balance, but also in nitrogenous waste excretion, such as ammonia and urea (Shimpei *et al.*, 2008).

The monogeneans are common ectoparasites with direct life cycles mainly parasitizing fish gills and fins (Jiri *et al.*, 2004). The impact of monogenean on fishes can be seen only when their number increase (excessive load) due to poor water quality in the aquatic medium (Reed *et al.*, 2009). Gill structural changes in response to monogenea in relation to water quality effects have been

rarely studied in fishes particularly in this part of Southeast Asia (Malaysia). Therefore this paper strives to address this issue which would throw light to the extent of gill damage caused by monogeneans coupled with poor environmental condition.

MATERIALS AND METHODS

Three hundred and eighty fish host (*Hemibagrus nemurus*) with mean weight 250 ± 0.5 g and standard length 23 ± 0.4 cm were collected from an earthen pond at Aquaculture Extension Center, Perlak, Pahang: Malaysia; using drag net (2.5" mesh). The study was conducted for a period of three months (Nov. 2010-Jan. 2011). The fish were brought to laboratory for examination. The gills were excised (both left and right sides) and placed in separate petri dishes with saline solution. Gill arches were rinsed with normal saline solution and fixed in 3% glutaraldehyde for Scanning Electron Microscopy (SEM) while others were fixed in bouin's solution for histology. Standard histological procedure described by Ogundiran *et al.* (2009) was followed.

Gills were removed from bouin's solution and replaced with 10% buffered formalin later transferred to

Table 1: Prevalence and mean intensity of gill monogenean and water quality parameters in *Hemibagrus nemurus*'s pond at Perlok (Pahang) for the months of November, December 2010 and January 2011

Parameters									
Months	Prev. %	Mean intensity	Temp.	DO	pH	TAN.	NH ₃	NO ₂	ALK
Nov.	76±2.1	18±1.2	23.1±0.50	7.8±0.2	6.7±0.3	2.101±0.20	0.005±0.002	0.003±0.001	148±25.4
Dec.	87±1.1	20±1.0	26.4±0.51	6.9±0.41	7.7±0.3	3.051±0.11	0.093±0.01	0.007±0.001	150±30.1
Jan.	82±1.0	13±0.9	25.5±1.30	5.8±0.78	7.1±0.21	2.125±0.07	0.016±0.02	0.002±0.10	410±9.27

Prev.: Prevalence; Temp.: Temperature (°C); DO (mg/L): Dissolved oxygen (mg/L) Tan.: Total Ammonia Nitrogen (mg/L); NH₃: Unionised ammonia (mg/L); NO₂: Nitrite (mg/L); ALK.: Alkalinity (mg/L CaCO₃)

Table 2: Showing pearson correlation matrix between monogenean infestation and different water quality parameters in *Hemibagrus nemurus* pond at perlok, pahang

	Prev. %	MI	Temp. °C	pH	DO mg/L	TAN mg/L	UN-NH ₃ mg/L	NO ₂ mg/L	ALK. /L mg/L CaCO ₃
Prev. %									
MI	0.227*								
Temp. °C	0.979**	0.024*							
pH	0.986**	0.386*	0.931**						
DO mg/L	-0.495	0.734**	-0.661	-0.344					
TAN mg/L	0.850**	0.705**	0.726**	0.926**	0.035				
UN-NH ₃ mg/L	0.895**	0.636*	0.787**	0.957**	-0.058	0.996**			
NO ₂ mg/L	0.721**	0.839**	0.565**	0.826**	0.245	0.974**	0.954**		
ALK. mg/L	0.059*	-0.959	0.260*	-0.108	-0.896*	-0.475	-0.391	-0.649	

**: Correlation is Significant at the 0.05 level (2-tailed); *: Correlation is significant at the 0.01 level (2-tailed)

tissue processor to dehydrate in a graded series of ethanol concentration up to absolute ethanol, infiltrated in xylene and embedded in paraffin wax. The gill in blocks were then cut into 5 µm sections and stained with haematoxylin and eosin (H&E).

For scanning electron microscopy study, gills were processed in the following procedure: dehydrated in series of increasing grade ethanol 35-100% later gills were subjected to critical point drying, using liquid CO₂. Specimens were then mounted on stubs and coated with gold in a sputter coater. Finally specimens were observed on SEM (JOEL, JSM 6360 LA).

Physical water quality parameters namely; dissolved oxygen (DO), pH, and temperature were measured instantly from the pond by using YSI meter (Model 556). While other parameters (chemical) such as total ammonia nitrogen (TAN), nitrite (NO₂⁻) and alkalinity were determined in laboratory from water sample collected from the pond. Un-ionised ammonia was later calculated from TAN in respect to values from pH and temperature. The parasitological terms prevalence, intensity and mean intensity follow definition by Bush *et al.* (1997).

Data recorded from all fish were analysed for prevalence and mean intensity. Pearson correlation matrix was calculated using SPSS 16 to show relationship between physico-chemical parameters with parasite infestation on the fish host.

RESULTS AND DISCUSSION

In the present study, prevalence of monogenean in the pond for the months of November, December 2010, and January 2011 were recorded as 76%±2.1, 87%±1.1 and 82%±1.0, respectively (Table 1). Infection rate per

months of investigation was 18±1.2 in November, 20±1.0 in December 2010 and 13±0.9 in January 2011 in that order. In December, an incidence of 20 worms on an individual fish has been recovered (Table 1). Statistical analyses have shown that there is a positive correlation between mean intensity and prevalence of parasites with water quality parameters namely temperature, ammonia levels, pH, dissolved oxygen and alkalinity (Table 2).

In the correlation matrix (Table 2), there are some perfect significant correlations among the water quality parameters (values with **). For example there is significant ($p<0.05$) correlation between temperature and monogenean prevalence. This gives rise to significant level of infestation in the pond with prevalence not less than 60%. Furthermore, mean intensity had perfect correlation with Un-ionized ammonia. The safe level of ammonia for freshwater fishes is 0.05 mg/L (Svobodova *et al.*, 1993; Floyd-F *et al.*, 2009). The result has indicated that, the ammonia concentration in the pond was exceptionally high in the month of December than November and January (Table 1). Higher level of ammonia in the pond might indicate level of pollution in the pond which in turn affects fish in the culture pond.

Nitrite according to Kroupova *et al.* (2005) is a natural component of nitrogen cycle in ecosystems and its presence in aquatic medium is a potential problem to organism (fish) living in that medium. The concentration of nitrite measured in the pond was 0.002 mg/L at minimum and 0.007 mg/L at maximum. However, according to Koskivaara (1992) acceptable level of nitrite in freshwater fish pond should range between 0.001-0.004 mg/L.

Alkalinity, which is the parameter responsible for buffering the aquatic media (Boyd, 1982) shows

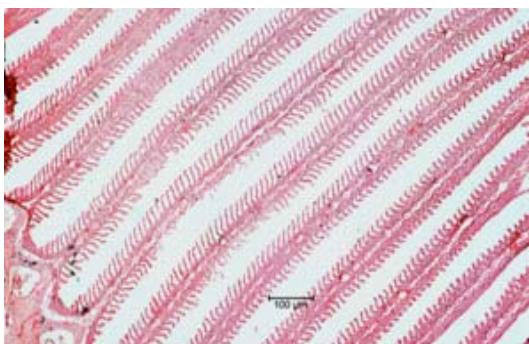


Fig. 1: A histological section of *Hemibagrus nemurus* gill free from monogenea parasites and water quality effects (H&E)

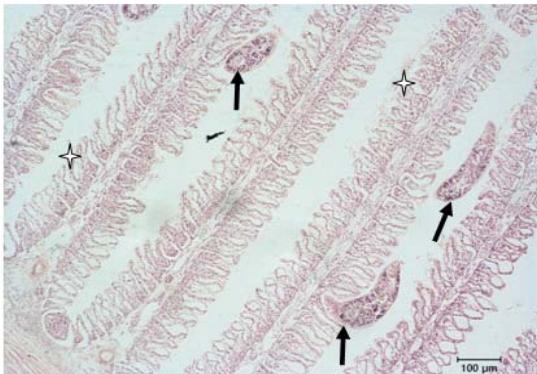


Fig. 2: A histological section through a gill of *Hemibagrus nemurus* showing mass attachment of monogeneans (arrows) on gill filament causing fusion and necrotic conditions (stars) in the gill filaments (H&E)



Fig. 3: Histological section of gill of *Hemibagrus nemurus* showing a telangiectasia condition at the tip of secondary lamellae (arrow)

statistically significant correlation ($p<0.05$) (Table 2) principally with temperature which is the co-determinant of most of the water quality parameters (Boyd, 1982; Koskivaara, 1992). The highest alkalinity recorded in the pond was 410 mg/L CaCO_3 , according to Boyd (1982)

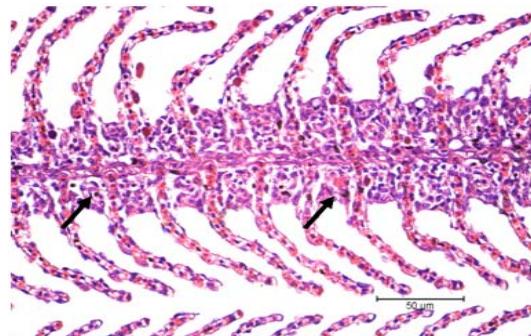


Fig. 4: Light micrograph of gill filament of *Hemibagrus nemurus* showing few number of chloride cells (see arrows) due to poor water quality condition of the pond (H & E)

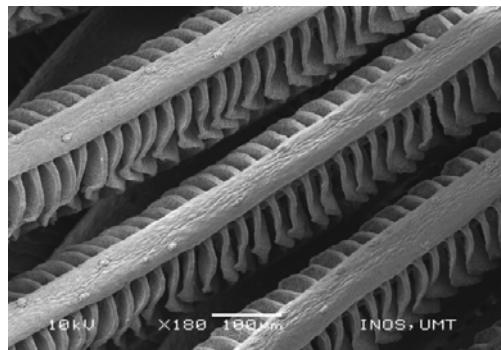


Fig. 5: A scanned micrograph of healthy gill filament of *Hemibagrus nemurus* showing devoid of monogenea and no water quality effects in PPA, Perlok fish pond

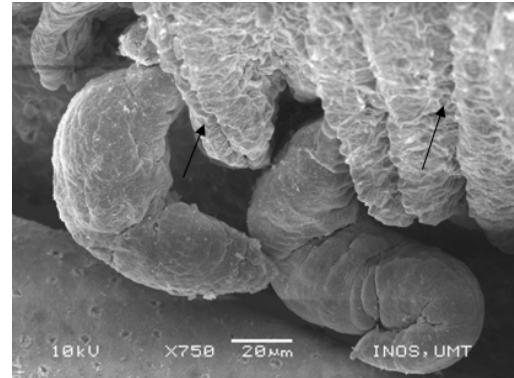


Fig. 6: A scanned micrograph of gill filament of *Hemibagrus nemurus* showing two monogeneans attached at secondary lamellae causing fusion (arrows) of the lamellae

can support phytoplankton development which in turn increase the level of zooplanktons in the pond.

The dissolved oxygen concentration in the fish pond ranged between 5.8-7.8 mg/L at temperature range between 23.1-26.4°C which is within the safety value for

freshwater cat fishes in the tropics (Koskivaara, 1992; Molnar, 1994; Chapman *et al.*, 2000; Bayoumy *et al.*, 2008).

Histopathological results revealed that in control fish gill (i.e., fish free from monogenea and water quality effects), primary lamellae appeared normal and mucus free with well defined secondary lamellae-no fusion or any hyperplasia condition observed (Fig. 1). In other fishes observed, effects of monogenea irritation and poor water quality deterioration shown various level of lesion.

Pathological alterations observed include proliferative, degenerative and necrotic changes in the epithelium of gill filaments and secondary lamellae, telangiectasia in secondary lamellae, haemorrhages on the gill filaments and mucous cells proliferation. Figure 2 shows mass attachment of monogeneans (arrows) on gill filament causing fusion and necrotic conditions (stars) in the gill filaments. In Fig. 3, a telangiectasia condition at the tip of secondary lamellae-atypical epithelia cells proliferation (arrows) and monogenea was also seen between the two lamellae.

From the scanning micrograph of healthy fish in the same pond, the gill filaments appeared to have no single monogenea attachment nor was any sign of water quality effects observed (Fig. 5). Two monogeneans attached at secondary lamellae (arrows) causing fusion of the lamellae (Fig. 6). This lesion condition in the present case could be a defence response by the fish as stated in the past by Morgan and Tovell (1973). However, the lamellar fusion could also be a protection device so that vulnerable gill surface areas are being covered. A few number of chloride cells were observed (Fig. 4) in the gill of the examined fish due poor water quality. According to Perry (1997), chloride cells or mitochondria-rich cells of the fish gills perform an integral role in acid-base regulation. In the present study, proliferation of the chloride cells might be due to elevated concentration of ammonia and nitrite in the pond water. This response reduces the number of functional $\text{Cl}^-/\text{HCO}_3^-$ exchangers particularly during alkalosis condition (Perry, 1997). A similar effect of nitrite in the gills of juvenile Amazonian fish (*Colossoma macropomum*) conducted by Ferreira da Costa and Fernandes (2002) have shown an Ultrastructural changes in the chloride cell indicating cellular functional disruption caused by exposure to nitrite.

Any change in the water quality is rapidly reflected in fish gill structure and function, since gills are continuously exposed to ambient water (Chezhian *et al.*, 2010). According to Erkmen and Kolankaya (2000), the ammonium concentration level higher than 1 mg/L indicates the considerable level of water pollution. Concentration of ammonia recorded in this study was 3.015 mg/L at maximum and 2.101 mg/L minimum

(Table 1). Excess ammonia may accumulate in gill filaments and causes alteration of metabolism or increase in blood pH. In the past, experimental effects of ammonia on various fishes have been carried out by many researchers (Avella *et al.*, 1989; Erkmen and Kolankaya, 2000; Frances *et al.*, 2000; Benli *et al.*, 2008) and found that exposure to un-ionized ammonia concentrations as low as 0.002 mg/L for few days of freshwater fish can causes hyperplasia of gill lining and further lead to secondary infection like bacteria and fungus.

CONCLUSION

In the present study, an elevated level of ammonia (3.051 mg/L) from the pond which is beyond acceptable level (0.005-1.00 mg/L) induces most of the structural changes in the gills apparatus. High prevalence and monogeneans intensity have also resulted the proliferation and abnormal changes on the gill filaments. Poor water quality condition in the pond does not only support monogenean loading but also other microbes. It can be concluded that gill alterations as a result of monogeneans irritation and poor water quality in fish ponds may serve as a sensitive biomarker for pollutants.

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