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Monogenean Parasites as Bio-indicator for Water Quality Status in Two Cultured Pond Fish Species in Perlok, Malaysia

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ABSTRACT
Study on the monogenean parasites as a bio-indicator for water quality in two fish ponds was conducted between 2010 and 2011 using standard parasitological Techniques. A total of 1000 fish comprises of 500 *Hampala macrolepidota* Van Hasselt and Kuhl 1823 and 500 *Hemibagrus nemurus* Valenciennes 1840 were sampled and examined from 0.2 ha fish pond in Perlok, Malaysia. Five monogenean species were detected from the gills of the examined fishes. They include; *Dactylogyrus macrolepidoti, Dactylogyrus quadribrachiatus, Dactylogyrus hampali* from *Hampala macrolepidota* while *Cornudiscoides malayensis* and *Cornudiscoides sundanensis* are from *Hemibagrus nemurus*. The results revealed that, incidence of the parasite infection was higher (100%) during non-monsoon (March – July) and the lower (44%) during monsoon (November – February) on both fish species. A high positive significant correlation ($P < 0.05$) was observed between the water temperature and monogenean prevalence. However, other water quality parameters such as ammonia, nitrite, pH and dissolved oxygen had also showed some significant relationship with the monogenean infection. The study concluded that monogenean infestation was enhanced by the water temperature and other factors of the water quality parameters, thus showed the indication character of the parasites in the aquatic environment.

Key words: Monogeneans, Bio-indicator, Monsoon, Non-monsoon, Water quality

INTRODUCTION

Monogeneans are commonly known as flatworm's parasites of fish inhibiting the external features of the fish (Skin and gills). They have a direct life cycle; proliferate either by live bearing species (*Gyrodactylus*) or egg producing species (*Dactylogyrus*) (Paperna, 1996). Several researchers recognized monogenean parasites as useful bioindicators for environmental pollution (Lafferty, 1997; Marcogliese and Cone, 1997; Landsberg *et al*., 1998; Galli *et al*., 2001; Marcogliese, 2005; Sanchez-Ramirez *et al*., 2007). Their occurrence or abundance can describe the situation of the environment (Sanchez-Ramirez *et al*., 2007; Palm and Ruckert, 2009). Bioindicators organisms are those species that reflect environmental impact because they respond to habitat alterations with changes in physiology or chemical composition (Vidal-Martinez *et al*., 2009). Moreover, bioindicators can be either accumulation or effect indicators (Sures, 2004). Monogeneans tend to increase in number when subjected to low and medium pollutant concentrations, but decrease or disappear at high concentration (Marcogliese *et al*., 1998). In Malaysia, basically two season are carefully observed, monsoon (heavy rainy season) and non-monsoon (dry or hot season) (Rouf, 2010). These seasons have directly or indirectly influenced the proliferation of monogeneans (Modu *et al*., 2012). The objectives of the study were to establish the relationship between monogenean parasitic infestation and water quality parameters of the ponds as it relates to seasonal variation during the period of the investigation.

MATERIALS AND METHODS

The study site

Aquaculture Extension Centre (AEC, Perlok) is located at Jerantut (3^{o} 56^{11} 48.62'' N, 102^{o} 22^{11} 47.5'' E) in Pahang, Malaysia (Fig. 1 A). It is a state owned fisheries development centre for the purpose of

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producing fish fingerlings and supply to commercial and other fish culturist in and around the state. AEC, Perlok, have more than 54 earthen fish ponds of various sizes and some concrete tanks in two different hatcheries (Fig. 1 B). The ponds were fed by stream as its water source. Virtually water coming from this stream was clean and void of most predators and parasites but due to culture intensity, some build-up of pathogens may be obvious. In the aquaculture centre, the fish were fed with high protein food supplements to increase productivity. Two ponds were extensively studied in this work. Pond 'A' contained *Hampala macrolepidota* while Pond 'B' contained *Hemibagrus nemurus*.

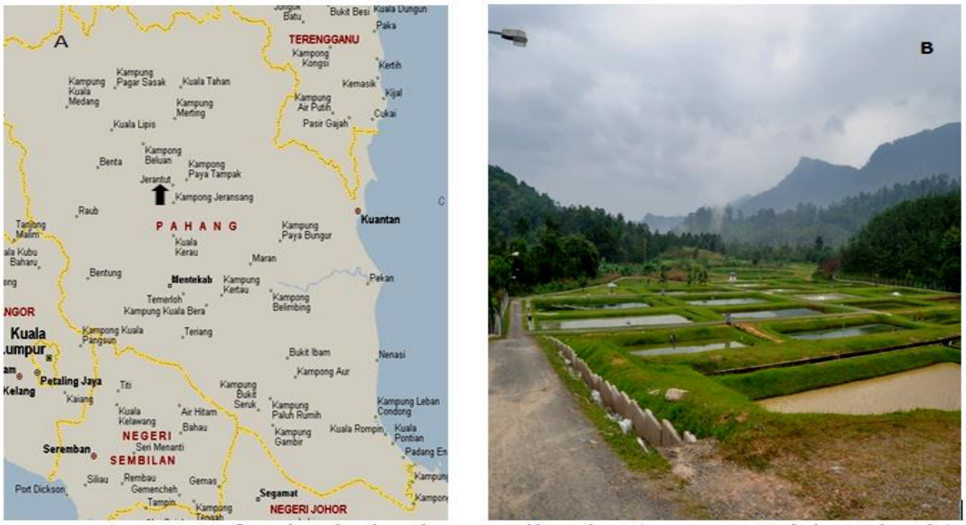

Figure: 1 A, Map of Malaysia showing sampling sites (Jerantut- Perlok, Malaysia); **B**, Ponds used during the study. (Source Google map)

Fish sampling and Laboratory examinations

A total of 1000 fish which comprised of 500 *Hampala macrolepidota* Van Hasselt and Kuhl 1823 and 500 *Hemibagrus nemurus* Valenciennes 1840 were sampled and examined from 0.2 ha fish ponds in Perlok, Malaysia. The fish were captured using seine net (mesh size 2 inch). They were brought in an aerated plastic bucket with pond water to the laboratory. They were killed by pithing using scalpel according to Berland (2005). They were measured and the values were recorded according to the method described by Fernando *et al*. (1972). The gills were excised immediately after death and placed in separate Petri dishes containing saline water. Each gill was examined for parasites and parasites found were mounted on clean glass slides. Mounted specimens were then observed under light microscope equipped with phase contrast optics (Nikon – Eclipse E200) for measurement of hard parts and reproductive organs for identification. A standard method described by Berland, (2005); Řehulková and Gelnar, (2006) was adopted for the parasites identification.

Water quality analysis

Water quality parameters, namely; Temperature, Dissolve Oxygen (DO), pH, Salinity Nitrite, Nitrate, and Ammonia were all measured according to APHA (1985). The parameters like temperature, DO, pH and salinity were determined directly in the ponds suing YSI 556 meter; while the ammonia, nitrate and nitrite were determined in the laboratory using HACH kit (Model: DR2400).

Data analysis

The level of parasites infections was quantified by the terms defined by Bush *et al*. (1997). Thus, the percentage of infection (Prevalence) is expressed as number of fish infected by individual species of parasite divided by number of fish examined multiplied by 100; while the Mean Intensity (MI) is total number of parasites divided by number of fish infected. Correlation between monogenean infestation and water quality was determined using Pearson correlation coefficient with SPSS 16 software. A pair sample statistical t-test was used to compare the means among water quality parameters of the two ponds.

RESULTS

Five known species of gill monogenean parasites were identified from two fish species (*Hampala macrolepidota* and *Hemibagrus nemurus*) from the farm. They include: *Dactylogyrus hampali*, *Dactylogyrus macrolepidoti, and Dactylogyrus quadribrachiatus*, from *Hampala macrolepidota*; *Cornudiscoides malayensis*, and *Cornudiscoides sundanensis* from *Hemibagrus nemurus*. The present results revealed that incidence of the parasite infestation was higher during non-monsoon (March – July) and the lower during monsoon (November – February) on both fish species from both ponds (Table 1) thus, water temperature varies with the atmospheric temperature i.e. being maximum during non-monsoon and minimum during monsoon.

The fluctuations in water temperature in both fish ponds are somehow similar. Pond 'A' had the lowest mean water temperature value compared to Pond 'B'. However, both ponds have similar maximum water temperatures (Table 1). The lower values of water temperatures were recorded during the monsoon period (November to January) while the higher values were recorded during non-monsoon (March to July) from both ponds. Monogenean prevalence in these fish ponds varied. In Pond 'A', high prevalence (> 90%) was observed at higher temperatures (> 25^{O}C) and moderate prevalence (≥ 50%) was recorded at moderate temperatures while low prevalence (≤ 40%) was noticed at lower temperatures (≥ 25^{O}C) (Figure 2). Worms' fluctuation in pond 'B' showed higher prevalence at higher temperatures (≥ 29 ^{O}C) and moderate temperatures supported moderate monogenean prevalence while low monogenean prevalence was found at moderate water temperatures (Figure 3).

Table: 1. Prevalence and mean intensity for five monogeneans in comparison with water temperature in two ponds (November 2010 – December 2011).

Fish species	***Hampala Macrolepidota* (N = 500) Pond A**						***Hemibagrus nemurus* (N = 500) Pond B**				**Water T^{O}C**	
Monogenean species	**DH**		**DM**		**DQ**		**CM**		**CS**		**Pond A**	**Pond B**
Date	**P%**	**MI**	**P%**	**MI**	**P%**	**MI**	**P%**	**MI**	**P%**	**MI**		
Nov.10	49	13	47	9	60	7	60	11	40	2	24.2	24.8
Dec.10	45	9	56	7	55	3	55	10	48	2	27.5	28.8
Jan.11	48	11	53	10	45	3	48	7	45	3	22.6	25.4
Feb.11	75	18	75	10	63	0	75	12	65	6	28.6	26.8
Mar.11	76	18	80	11	60	8	68	9	60	4	30.1	30.2
Apr.11	80	14	80	7	35	7	80	15	88	11	27.3	29.3
May.11	83	29	83	19	56	11	95	23	85	7	26.3	25.7
Jun.11	82	9	85	21	75	7	89	19	75	14	30.1	29.9
Jul.11	100	10	100	36	60	3	95	27	85	16	31.9	30.1
Aug.11	53	17	56	11	26	0	20	4	20	5	24.9	25.4
Sep.11	48	12	51	7	36	5	54	9	48	6	26.8	25.6
Oct.11	90	33	100	32	56	0	85	16	68	11	26.7	28.6
Nov.11	48	26	45	6	54	11	50	8	43	5	26.1	27.3
Dec.11	44	31	55	11	26	0	45	5	35	3	25.3	25.2

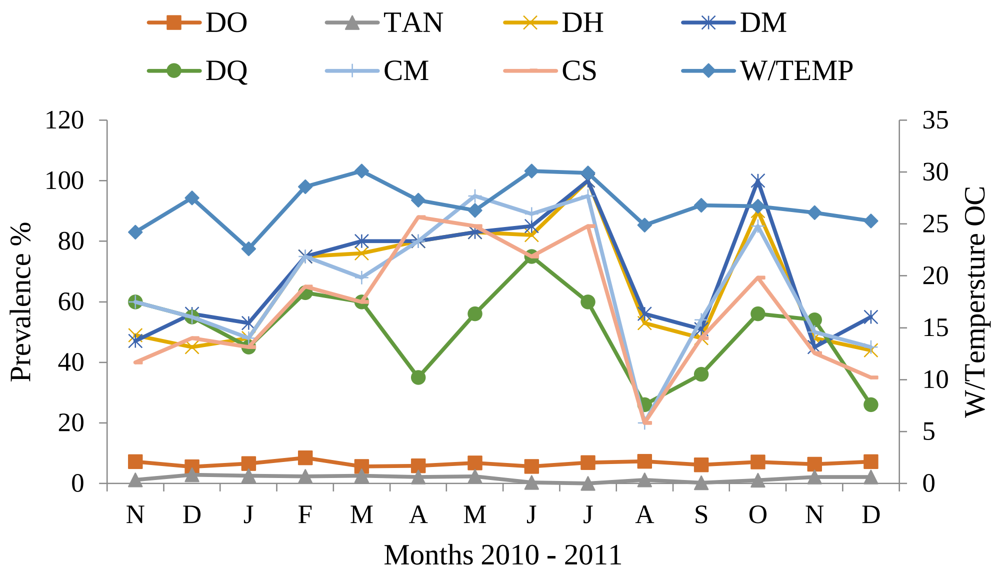

Fig: 2. Temperature patterns in pond A

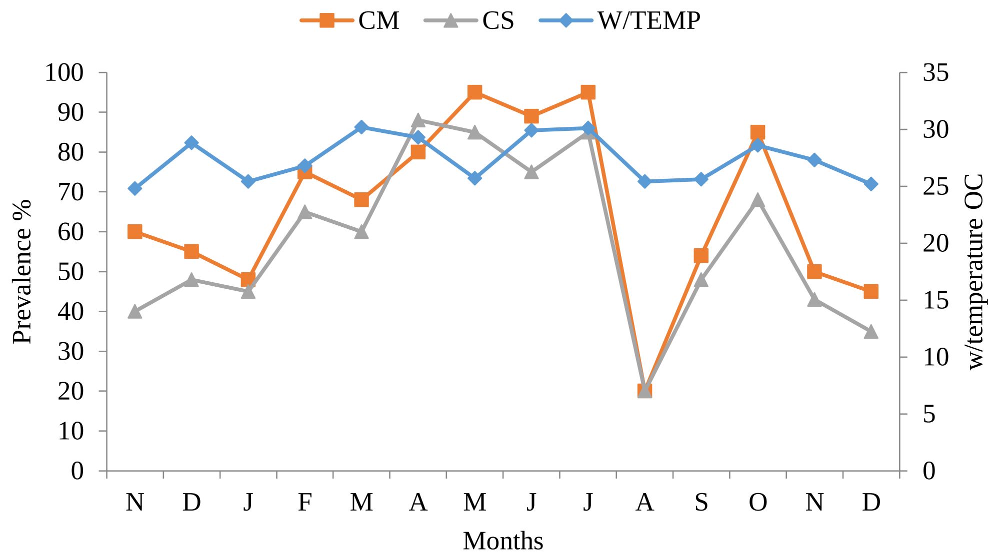

Fig: 3. Temperature patterns in pond B

Correlation between parasites prevalence and water quality parameters

The ponds water temperature showed apparently a perfect correlation in all cases of monogenean prevalence on both fish species investigated (P<0.01) (Table 2). The ponds water pH had little or no significant influence(P>0.05) over the parasites proliferation. The dissolved oxygen of the ponds water also showed little relations with most cases of the monogeneans except a weak negative association was observed between the ponds DO with *D. quadribrachiatus* in pond 'A'. Total ammonia-nitrogen (TAN) in pond 'A' showed a weak negative relation with the prevalence of *D. hampali* and *D. macrolepidoti* (r = -0.326, r = -0.290) while with *D. quadribrachiatus* a weak positive correlation was observed (r = 0.224) (Table 2). In contrast to pond 'B', the pond water TAN had a strong negative correlation with *C. malayensis* and *C. sundanensis* (r = -0.239 and r = -0.160). Nitrite-nitrogen (NO_2-N) on the other had a weak positive significant with *D. hampali* and *D. macrolepidoti* in pond 'A' and similar relation was noticed in pond 'B' with *C. malayensis* and *C. sundanensis*. A perfect negative non-significant relation was observed between un-ionized ammonia with all cases of monogenean species (P>0.05) in both fish ponds. Total alkalinity of both ponds water showed weak positive relation with regards to monogeneans prevalence, a similar relationship was also observed with water transparency. Nitrate-nitrogen (NO_3-N) of the ponds water showed weak negative relation with two monogenean species i.e. with *D. hampali* and *D. macrolepidoti* on *H. macrolepidota* while weak positive association was observed with *D. quadribrachiatus C. malayensis* on *H. macrolepidota* and *H. nemurus* respectively.

Table: 2. Pearson correlation matrix between prevalence of five gill monogenean species and water quality parameters investigated in two fish ponds.

Fish species	***Hampala macrolepidota* (Pond 'A')**			***Hemibagrus nemurus* (Pond 'B')**	
Monogenean species Parameters	**DH**	**DM**	**DQ**	**CM**	**CS**
$T^{O}C$	0.764**	0.698**	0.889**	0.659**	0.660**
pH	-0.162	-0.168	0.062	-0.098	-0.073
DO (mg/L)	0.191	0.152	-0.501	-0.118	-0.214
TAN (mg/L)	-0.326	-0.29	0.224	-0.239	-0.16
NO_2-N (mg/L)	0.241	0.245	-0.221	0.11	0.09
NH_3-N (mg/L)	-0.098	-0.107	-0.106	-0.103	-0.099
ALK. (mg/L $CaCO_3$)	0.198	0.145	0.423	0.416	0.39
TRANSP. (m)	0.381	0.335	-0.046	0.195	0.288
NO_3-N (mg/L)	-0.24	-0.292	0.296	0.054	-0.16

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 (2-tailed). **Monogenean species**: DH = *Dactylogyrus hampali,* DM = *Dactylogyrus macrolepidota,* DQ = *Dactylogyrus quadribrachiatus,* CM = *Cornudiscoides malayensis,* CS = *Cornudiscoides sundanensis.*

Key: T. = Temperature, DO = Dissolved oxygen, TAN = Total ammonia-nitrogen, NO_2-N = Nitrite-Nitrogen, NH_3-N = Unionized ammonia, ALK. = Alkalinity, TRANSP. = Transparency, NO_3-N = Nitrate-Nitrogen. **Note: values shown here are correlation coefficient 'r' values intercept with corresponding variables.**

DISCUSSION

The impact of environmental factors on monogenean communities have been stressed by many authors (Koskivaara, 1992; Mackenzie *et al*., 1995; Galli *et al*., 2001; Sures 2004; Marcogliese 2005; Hudson *et al*., 2006; Bayoumy *et al*., 2008) and found that several factors including water temperature, dissolved oxygen, pH, and total ammonia-nitrogen could influence monogenean proliferation in different fish species. In this study, water chemistry investigated from the two ponds in relation to monogenean prevalence has revealed some considerable changes in monogenean infestation among the two fish species examined. Very few studies have considered interaction between monogenean and water quality parameters especially in field-based study. For example, Koskivaara *et al*. (1991) demonstrated a correlation between Gyrodactylids diversity with roach and water quality. Bayoumy *et al*. (2008) correlated five monogenean species with water temperature and heavy metals in some Egyptian Rea Sea fishes and confirmed that water quality especially temperature enhanced monogenean life cycle.

A rise in water temperature accelerates chemical reaction, reduces the solubility of gases, amplifies test and odour, and elevates the metabolic activity of organisms (Chandrasekhar, 2006). The water temperatures observed in these ponds were considerably lower when compared to natural water bodies in the region (e.g. Kenyir Lake). The maximum water temperature recorded in pond 'A' and pond 'B' were 31.9 and 30.2^{O}C while the highest temperature of Kenyir Lake was 32.83^{O}C (Modu *et al*., 2012). This variation may be attributed to several factors such as human activity, pond inputs like feeds and fertilizers. Ponds in the study site were mechanically aerated to increase oxygen content and such process can easily decrease the water temperature, as the pond water bubbles the free atmospheric oxygen is being mixed thereby increasing the oxygen content in the pond water, conversely it may influence the proliferation of monogeneans (Bayoumy *et al.*, 2008). In all cases of monogenean species investigated, prevalence was higher during non-monsoon (April to July) period and lower during monsoon (November to February). According to Boyd and Tucker, (1998), seasonal pond water temperature changes may impair immune function of fish, even if changes occur within the range considered optimal.

The pH values recorded in pond ‘A’ and pond ‘B’ were not similar. Pond ‘A’ is an alkaline pond as observed in the water quality analysis (pH >9). This pond had some vegetation cover, and photosynthetic processes possibly added some nutrients, while death and decay of plants might have enhanced build-up of microbes which in turn changed the water chemistry. pH in pond ‘B’ did not reach 8.5 and hence considered as ideal pond for the stocked cat fish to survive. In both ponds, relation between pH and five gill monogeneans prevalence are not significant (P>0.05).

The dissolved (DO) concentration recorded in the ponds were within the safe level (range 5.5 – 8.4 mg/L) as suggested by Svobodova *et al*. (1993). During the non-monsoon DO tend to be low due to increase in metabolic activity and diurnal fluctuation due to respiration. At this season, aeration activity was observed in both fish ponds because it is the only possible means to increase the DO level in the ponds. Monogenean prevalence at this time was observed to be higher. However, this condition usually favours monogenean infestation. But any deviation from this act or depletion in DO and sudden decrease in water temperature tend to reverse the condition (Bauer *et al*., 1973). Unlike their temperate counterpart, tropical monogeneans can easily proliferate faster during high DO and high temperature period. This situation was clearly observed in this work, where four among the five monogenean species recovered (*D. hampali*, *D. macrolepidota*, *C. malayensis* and *C. sundanensis*) from two fish species examined at AEC, Perlok showed high peak of infestation (> 75%) during non-monsoon period (Table 1). At monsoon period constant rain and air turbulent enhanced solubility of free oxygen to the ponds thereby increase the level of DO.

Total ammonia-nitrogen (TAN) in aquatic ecosystem is a by-product of fish protein metabolism and bacterial decomposition of organic matter (Francis-Floyd *et al*., 2009). TAN is the combined measures of its two forms; un-ionized ammonia (NH_3-N) and ammonium ion (NH4+). The decay of uneaten food and organic matter create small amounts of ammonia, but in most aquaculture systems, fish themselves are the primary source of the compound. The more feed a fish receives, the more ammonia it will produce (Francis-Floyd *et al*., 2009). The concentrations of TAN observed in both fish ponds are subject to the increase in the pond water temperature and pH as suggested by Boyd (1982). In the present study, the results showed that concentrations of TAN and Un-ionized ammonia in pond ‘A’ (Hampala pond) increased as pond water temperature and pH increased. This condition might influence monogenean proliferation and in certain instance it might reduce their population especially during the non-monsoon period. According to Colt and Armstrong (1979), as ammonia level increases in water, ammonia excretion by fish decreases and levels of ammonia in blood and tissues increases. Boyd (1982) added that the outcome of such situation can adversely affect enzyme-catalysed reactions and membrane stability, thus fish immune capability reduces (Perpana, 1996) which favour parasites and other microbes to build up in the affected fish. In general, according to Boyd and Tucker (1998), the rate of ammonia production in a cultured pond is proportional to the feeding rate. In a nutshell, about 0.03 Kg of ammonia-nitrogen is excreted by fish per 1kg of high quality (25 – 40% crude protein) feed consumed (Boyd and Tucker, 1998). Elevated levels of ammonia in the ponds at AEC, Perlok presented in this study might be due to the postulated theory by Boyd and Tucker, (1998) in that fish from this organization are apparently fed with high quality feed supplement (45% crude protein).

Conclusion

Finally, the results of the present study showed that there are significant correlations between all species of monogenean parasites and water temperature from both ponds (P < 0.05). This condition was observed where monogenean prevalence and intensities were more pronounced at higher temperatures (> 29^{O}C), particularly during the non-monsoon (dry season; March to July) in contrast to those observed during monsoon time (November to January) in both fish species. The present finding also statistically justified that the water temperature and dissolved oxygen are the two major water quality parameters influencing the proliferations of most of the monogenean species recovered on the two fish species investigated.

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