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SPATIAL DISTRIBUTION OF MONOGENEANS AND THEIR PREVALENCE IN TWO FRESHWATER FISH SPECIES IN RELATION TO SOME PHYSICO-CHEMICAL PARAMETERS OF KENYIR LAKE, MALAYSIA

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Abstract: A parasitological investigation was carried out on two freshwater fish species – *Hampala macrolepidota* and *Hemibargrus nemurus* (n = 460) for gill monogeneans from four stations in Kenyir Lake [station I–Kiang River (5˚6'49.47" N, 102˚44'36.60" E); station II – Petang Island (4˚58'35.65" N, 102˚47'3.74" E), station III –Ketiar River (5˚3'48.38" N, 102˚34'14.70" E) and station IV -Tanjung Mentong (4˚48'36.54" N, 102˚45'40.56" E)]. Four monogenean parasites were detected viz: *Bifurcohaptor baungi* and *Cornudiscoides malayensis* from the gills of *Hemibargrus nemurus*; *Dactylogyrus quadribrachiatus* and *D. macrolepidoti* from gills of *Hampala macrolepidota*. Their spatial distributions on the host gills are reported. The prevalences of these parasites were correlated to physico-chemical parameters (temperature, dissolved oxygen, pH, ammonia, nitrite, and alkalinity) of the lake water. The results revealed that all the stations were infected, but the highest infestation was in the third station (Ketiar River) with 100% prevalence and intensity of 43 individual worms per host. Correlation between physico-chemical parameters and parasites prevalence shows statistical significance ($P < 0.05$), especially between prevalence, mean intensity and total ammonia nitrogen (TAN). In conclusion, monogeneans from these two fish species were not randomly distributed but showed site specificity.

KEYWORDS: Prevalence, Mean intensity, Physico-Chemical parameters, *Bifurcohaptor baungi*, *Dactylogyrus quadribrachiatus*

Introduction

Monogeneans are mostly restricted not only to a particular host but also to a particular part of their host's body (Turgut *et al.,* 2006). However, Wootten, (1974) earlier observed that for the monogenean (*Dactylogyrus amphibothrium* Wagener, 1857) on the gills of ruffe (*Gymnocepalus cernua* L.) the spatial distribution was not random and that the worms were confined on certain parts of the gill basket.

Studies on the relation between different monogeneans inhabiting the same host and their distribution on the gills apparatus have been stressed by many authors (El Hafidi *et al.,* 1998, Dzika, 1999, Chapman *et al*., 2000; Simkova *et al*., 2000, Turgut *et al.,* 2006). According to Godoy-Rubio (2008), gill-infecting monogeneans commonly exhibit very precise site specificity on their host gills. For instance, *Pseudodactylogyrus anguillae* in contrast with *P. bini* are more frequently found on the second and third gill arches but certain parts of the gills are occupied by both species (Dzika, 1999). Shaharom (1985) in her study on *Cichlidogyrus sclerosus*, its juveniles and *C. tubicirrus minutus* on the gills of tilapia fish, found that both parasites preferred the first gill arches of the host fish. The first gill arch according to her finding was closer to the operculum and was considered to have least current flow. She further stressed that the subsequent arches (2, 3, & 4) possess a "fence mechanism" (microbranchiospines) which hindered the easy movement/attachment of the

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worms (especially the juveniles) and sieved the water passing through the gaps.

Monogeneans on these two species of fishes (*Hampala macrolepidota*, and *Hemibargrus nemurus*) have been described by Lim & Furtado (1983) and Lim (1987) based on their distribution and diversity from other parts of Peninsular Malaysia. However, little or less attention has been paid to site specification in contrast to water quality on the gill of their host at Kenyir Lake. This work presents a study on the spatial distribution of several species of monogeneans so that their preference for specific parts of the fish gills basket may be established. The study also aims at correlating the parasite intensity to some physico-chemical parameters (temperature, dissolved oxygen, pH, ammonia, nitrite, and alkalinity) of Kenyir Lake.

Materials and Methods

Study site

Kenyir Lake, an immense body of water situated in the interior of Terengganu state, is the largest man-made lake in South East Asia (Hasan and Ambak, 2005). At longitude 102° 35'- 102° 55' E and latitude 4° 40'- 4° 50' N, it is situated in the luxurious forest of Kenyir in Hulu Terengganu district. The lake serves as an important ecosystem for aquatic fauna and flora and also as a recreational centre for tourists. Fishing and angling are the major activities in the lake. The lake harbours many species of fish and shellfish which provide food and a recreational arena for the state populace.

Fish sampling

Fish specimens (*Hampala macrolepidota* weighing 10 -250g and *Hemibargrus nemurus* weighing 15 – 450g) were caught by gill netting (2.0 and 2.5 inch mesh) from four different locations (Kiang River, Petang Island, Ketiar River and Tanjung Mentong) along Kenyir Lake for a period of six months (January to June 2010). Fish caught were brought to the laboratory and kept in small aquaria containing aerated lake water.

Water-quality analysis

Physical parameters (dissolved oxygen, pH, and temperature) of the lake water were determined on board using YSI meter (Model 556), while chemical parameters such as ammonia (TAN), un-ionised ammonia, nitrite, and alkalinity were determined in the laboratory. Collected water samples were placed into bottles (500ml) and acidified with sulphuric acid (H_2SO_4) to stop further microbial activities before analysis. The physico-chemical parameters were measured once a month.

Parasitological examinations

Gills of four hundred and sixty (460) fish specimens from *Hampala macrolepidota* (n = 230) and *Hemibargrus nemurus* (n = 230) were examined according to the method described by Fernando, *et al* (1972). Fresh live fishes were killed by pithing and gills from both sides were excised and placed in separate petri-dishes containing saline water (Kaewviyudth and Chinabut, 1999). Monogeneans found on the gills were isolated from the mucus by sucking up with a modified glass pipette (Berland, 2005), transferred into a drop of water on a clean glass slide and scanned for the presences of worms. A cover slip was placed on top then four corners of the cover slip were glued with nail varnish to stop its motion. A drop of ammonium-picrate-glycerine was added to fix and clear the worm. Glycerine was added where necessary to remove any bubbles in the preparation process. Fixed specimens were then observed under a light microscope (Nikon – Eclipse E200, @ x 50 & 100 magnifications) for identification.

Gills arches from both sides of the fish were numbered I-IV from the anterior gill arch below the operculum to the posterior. The surface of each hemibranch was designated as outer (the surface nearer to the operculum) and inner, (Turgut *et al.*, 2006) and each gill arch was arbitrarily divided into six sections: 1, 2, 3, 4, 5, & 6, obtaining 24 sectors from four gill arches of one side (Shaharom, 1985; Dzika, 1999). The number of worms on each sector was collected and recorded with their positions as shown in the gill map (Figure 1).

Statistical analysis

The parasitological terms (prevalence, intensity and mean intensity) followed Bush *et al*. (1997) where the prevalence (P) is the number of fish with one or more individuals of a particular parasite divided by the number of hosts examined (expressed as a percentage) multiplied by one hundred. Intensity (of infection, I) is the number of a particular parasite species in a single host (expressed as numerical range). Mean intensity (of infection MI) is the average intensity, or the total number of a particular parasite species found in a sample divided by the number of infected hosts.

Data obtained were analysed to calculate the prevalence, and mean intensity and to correlate the relationship between physico-chemical parameters with a percentage of infestation on the hosts studied. All data were subjected to SPSS 16 statistical software to evaluate Pearson correlation between the environmental factors and parasite prevalence.

Results and Discussion

The prevalence and mean intensity (in parentheses) of monogenean parasites on fish from four different stations (Kiang River, Petang Island, Ketiar River and Tanjung Mentong) investigated were 53 ± 23.1% (9.0 ± 5.6), 94 ± 9.8% (19.0 ± 3.4), 100 ± 0.0% (43.0 ± 6.8) and 87 ± 11.5% (5.0 ± 3.3) respectively (Table 1).

Table 2 shows the correlation matrix between monogenean infestations in fish and some of the physico-chemical parameters studied. Significant ($P < 0.05$) correlations were observed between temperature, parasite prevalence, rate of infestation and other water-quality parameters.

The dissolved oxygen concentration recorded during the period of study ranged between 6.4 – 7.2 mg/L at temperatures ranging between 26.9 – 29.5^{O}C (Table 1) which is within the safety value for freshwater inhabitants in the tropics (Boyd, 1982; Paperna, 1991; Rottmann, *et al.* 1992; Molnar, 1994; Chapman, *et al.* 2000). Data from these sites showed that the fishes were infected in almost all the stations but higher infection was at the third station (Ketiar River), 100%. At the time of this survey, fresh water was coming from upstream and this may have initiated the accumulation of monogeneans as suggested by several authors (Wootten, 1974; Rhode, 1982; EL Hafidi, *et al*., 1998; Dzika, 1999; Turgut, *et al.,* 2006, and Godoy-Rubio, 2008) relating to amount of water passing over the gills of the host; thus, the volume of passing water may influence aerobic situation (Wootten, 1974) in the gill surface and hence facilitate more parasite settlements.

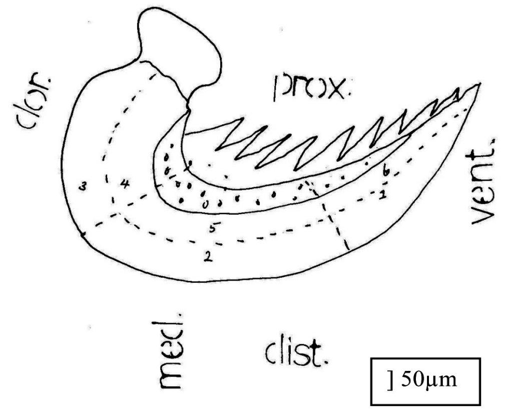

Figure 1: Schematic mapping of gill arch showing its division into six arbitrary areas: 1.distal-ventral, 2. distal-median, 3. distal-dorsal, 4. proximal-dorsal, 5. proximal-median, 6. proximal- ventral. *(*Modified after *Turgut et al. 2006).*

Despite the high prevalence in Ketiar River, the concentration of un-ionised ammonia was minimum. This may have been due to bicarbonates in the water which buffered the water. This was evident by the present record of alkalinity (138 mg/L $CaCO_3$) (see Table 1) which is within an acceptable range (50 - 300 mg/L $CaCO_3$) as suggested by Svobodova, *et al.* (1993). The lowest value of total alkalinity observed among the stations studied was in Kiang River (93 mg/L $CaCO_3$) while the highest was in Tanjung Mentong (222 mg/L $CaCO_3$). Highly significant ($P < 0.05$) correlations were detected between total ammonia nitrogen (TAN) and parasite prevalence at all the stations. The safe level of TAN in fresh water according to Svobodova, *et al.* (1993) and Floyd, *et al.* (2009) is 0.05mg/L.

Temperature showed variations for different study areas. The highest water temperature recorded was 32.3 ^{O}C in Tanjung Mentong while the lowest temperature 26.9 ^{O}C recorded was in Kiang River (Table 1). Temperature plays a very vital role in the manipulation and fluctuation of several abiotic parameters particularly in relation to monogenean life cycle (Koskivaara, 1992). In this study, positive significant correlations were observed between temperature and prevalence of the monogeneans infections (Table 2). Bayoumy *et al* (2008) reported that temperature is the most important abiotic parameter and affects parasites at all life-cycle stages.

The mean pH value of the lake water was found to be slightly varying from a minimum pH of 6.5 in Ketiar River to a maximum of 7.8 in Petang Island (Table 1).

Parasite identification

Four monogenean parasites were identified from the gills of the studied fishes. They included the following: *Dactylogyrus quadribrachiatus,* and *Dactylogyrus macrolepidoti,* from *Hampala macrolepidota*; *Cornudiscoides malayensis* and *Bifurcohaptor baungi* from *Hemibargrus nemurus* (Lim, & Furtado, 1983; Lim, 1987). Some common diagnostic features of these monogeneans may include the following:

1. All possess 4 eye spots.
2. All have anchor/hamuli for attachment.
3. *Dactylogyrus quadribrachiatus* has X-shape ventral bar.
4. *Dactylogyrus macrolepidoti* has thin and stick-like ventral bar.
5. *Cornudiscoides malayensis* has two pairs of hamuli of different sizes (small & big).
6. *Bifurcohaptor baungi* has bifurcated haptor with long dorsal hamuli.

Spatial distribution of monogeneans on gill apparatus of examined fishes

Monogeneans show a preference for the different parts of the gills of the host fishes (El Hafidi *et al*., 1998; Dzika, 1999; Shaharom, *et al.* 1985). In this study, all the monogeneans examined show preferences for particular branchial arches or certain parts of the gill apparatus. Table 3 and 4 show spatial distribution of four different monogenean species over the gills of their host fish. In Table 3, *Dactylogyrus quadribrachiatus* shows significant preference on outer hemibranch and distal –median ($P < 0.05$) of the third gill arch on both sides of the fish gill while *D. macrolepidoti* preferred distal – dorsal of the same outer hemibranch. This may have been due to strongest water flow that passes across this portion of the gill filaments (Wootten, 1974), thus creating convenient habitat for the settlement of the parasites (Turgut *et al*., 2006). Turgut *et al*. (2006) further reported that differences in water current over the different parts of gill surfaces have been considered important in determining the distribution of monogeneans on the host gills. There were significant differences in the number of *D. quadribrachiatus* on the different gill areas ($P < 0.05$). A greater number of them were found on the distal-median than the proximal - dorsal segments of the gill filament. The number of *D. macrolepidoti* on the different parts of gill filaments of *Hampala macrolepidota* is shown in Table 3. The data analysis showed statistical differences in the number of *D. macrolepidoti* between the gill arches. A considerable number of *D. macrolepidoti* occupied the third and fourth gill arches from both sides of the gill apparatus. A greater number of *D. macrolepidoti* was observed from the outer hemibranch of the gill filament than from the inner. Higher numbers of *D. macrolepidoti* were observed on the distal-median and distal-dorsal segments than on the proximal-dorsal and proximal median of the gill. It was observed that there was a mixed form of infestation in *Hampala macrolepidota* that shows there is mutual coexistence between *D. quadribrachiatus* and *D. macrolepidoti* in terms of niche sharing as in similar study carried out by Yang, *et al.* (2006) on *Polyabrs mamaevi* and *Tetrancistrum nebulosi* on the gills of *Siganus fuscescens.*

In this study, a greater number of *D. quadribrachiatus* and *D. macrolepidoti* were found attached to the outer rather than the inner face of gill hemibranch of *Hampala macrolepidota*. In this finding, it was found that most of the monogeneans were recovered on the third and fourth gill arches, in agreement with works of some authors who found the highest

Table 1: Prevalence, mean intensity and physico-chemical parameter variations in four stations investigated for gill monogeneans in Kenyir Lake. Mean (± SD)

Stations (I-IV)	Prev. %	MI	T ^{O}C	pH	DO mg/L	TAN mg/L	NH_3 mg/L	NO_2-**N** mg/L	Alkalinity mg/L $CaCO_3$
Kiang River	53 ±23.1	9 ±5.6	26.9 ±1.4	6.9 ±0.6	6.5 ±0.4	0.09 ±0.6	0.001 ±0.0	0.004 ±0.0	93 ±39.6
Petang Island	94 ±9.8	19 ±3.4	29.3 ±0.4	7.8 ±0.1	7.2 ±0.4	0.34 ±0.6	0.016 ±0.0	0.004 ±0.0	143 ±3.0
Ketiar River	100 ±0.0	43 ±6.8	29.8 ±2.2	7.8 ±0.3	6.5 ±0.7	1.35 ±0.4	0.064 ±0.0	0.015 ±0.0	138 ±49.9
Tanjung Mentong	87 ±11.5	5 ±3.3	32.2 ±0.3	7.5 ±0.1	6.4 ±0.2	0.37 ±0.5	0.003 ±0.0	0.002 ±0.0	222 ±121.7

(Prev. = prevalence; MI = mean intensity; T = temperature; DO = dissolved oxygen; TAN = total ammonia nitrogen; NH_3 = un-ionised ammonia; NO_2-N = nitrite-nitrogen)

Table 2: Pearson correlation matrix between the prevalence and mean intensity of monogeneans examined and other physico-chemical parameters of Kenyir Lake.

	Stations	Prev %	MI	T ^{O}C	pH	DO mg/L	TAN- mg/L	NH_3 mg/L	NO_2-N mg/L	Alkalinity mg/L $CaCO_3$
Stations										
Prev	.663									
	.337									
MI	.091	.607								
	.909	.393								
T	.974*	.669	-.041							
	.026	.331	.959							
pH	.548	.987*	.608	.575						
	.452	.013	.392	.425						
DO	-.349	.300	.074	-.183	.446					
	.651	.700	.926	.817	.554					
TAN	.428	.700	.927	.274	.646	-.189				
	.572	.300	.073	.726	.354	.811				
NH_3	.237	.649	.986*	.090	.625	-.052	.977*			
	.763	.351	.014	.910	.375	.948	.023			
NO_2	.109	.468	.966*	-.066	.439	-.175	.940	.976*		
	.891	.532	.034	.934	.561	.825	.060	.024		
Alk	.920	.509	-.265	.974*	.418	-.193	.056	-.135	-.281	
	.080	.491	.735	.026	.582	.807	.944	.865	.719	

(Prev. = prevalence; MI = mean intensity; T = temperature; DO = dissolved oxygen; TAN = total ammonia nitrogen; NH_3 = un-ionised ammonia; NO_2-N = nitrite-nitrogen, Alk =Alkalinity

***Correlation is significant at the 0.05 level (2-tailed)**

number of *Dactylogyrus* spp occurring on the same gill arches and lowest numbers in the first gill arch (Turgut, 2006; Wootten, 1974, Koskivaara *et al.,* 1992).

In *Hemibargrus nemurus*, few *B. baungi* were recovered and the spatial distribution on the gill filaments shows that *B. baungi* preferred the first gill arch and outer surfaces of the gills. Most of them were found on the distal-dorsal segments rather than proximal-ventral. *B. baungi* can be observed browsing its food towards inner segment of the gills while attached at dorsal surfaces in the live specimen. Preference of *B. baungi* for the first gill arch is perhaps related to its body size (1000µm). The number of *Cornudiscoides malayensis* on different parts of the gill apparatus is shown in Table 4. The data analysis did not show any significant differences in the number of *C. malayensis* between the left and right sets of the gill arches of *H. nemurus* (P> 0.05). *C. malayensis* predominantly occupied most parts of the gill segments in contrast with *B. baungi* in *H. nemurus*. *C. malayensis* preferred the inner hemibranch on the distal halves of the gill lamellae. Most of them were recovered at the median segment of the hemibranch.

Table 3: Spatial distribution of *Dactylogyrus quadribrachiatus* and *Dactylogyrus macrolepidoti* on the gill arches of *Hampala macrolepidota* (N = 230).

Aspect	*Dactylogyrus quadribrachiatus*		*Dactylogyrus macrolepidota*	
	Left	Right	Left	Right
Gill arch I	8	10	12	9
Gill arch II	32	16	18	19
Gill arch III	45	32	33	20
Gill arch IV	14	29	33	38
Dorsal segment	30	32	30	33
Median segment	32	40	38	31
Ventral segment	28	15	28	21
Proximal	44	42	32	33
Distal	55	45	64	53
Inner	46	38	39	34
Outer	53	49	57	52

Table 4: Spatial distribution of *Bifurcohaptor baungi* and *Cornudiscoides malayensis* on the gill arches of *Hemibagrus nemurus* (N = 230)

Aspect	*Bifurcohaptor baungi*		*Cornudiscoides malayensis*	
	Left	Right	Left	Right
Gill arch I	-	-	45	40
Gill arch II	01	-	67	76
Gill arch III	02	01	89	91
Gill arch IV	02	02	98	99
Dorsal segment	04	02	98	99
Median segment	01	01	117	106
Ventral segment	-	-	56	67
Proximal	02	-	104	95
Distal	03	03	167	177
Inner	-	-	161	172
Outer	05	03	110	100

Conclusion

Spatial distribution of these four monogeneans found on the two fish species indicated that all the monogeneans show preference for the outer rather than the inner surfaces of the gill lamella. However, each has a special preference on the different segments and different gill arches of the gill apparatus. From this investigation it is therefore concluded that monogeneans on the two fishes were not randomly distributed but showed site specificity. The correlation between physico-chemical parameters and parasite prevalence showed statistical significance ($P < 0.05$) especially between prevalence, mean intensity and total ammonia nitrogen (TAN), suggesting that changes in water-quality parameters may either increase or decrease parasite proliferation.

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