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RELATIONSHIP BETWEEN pH, WATER TEMPERATURE, DISSOLVED OXYGEN AND PARASITIC INFESTATION OF FRESHWATER FISHES IN TEMENGOR, BERSIA AND CHENDEROH RESERVOIRS, PERAK, MALAYSIA

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ABSTRACT
Fishes in the reservoirs are subjected to many parasitic diseases due to poor quality of water and large-scale mortality is seen in the fish population due to parasitic diseases. There were no documented reports relating to fish parasite interactions with abiotic factors of freshwater fish parasitism in Temengor, Bersia and Chenderoh reservoirs. Fish were examined for parasites using standard parasitological techniques. Three in-situ replicates of physico-chemical parameters were taken at each sampling period from the reservoirs. Dissolved oxygen (DO) concentration (mg/L), temperature (°C), and pH of the reservoirs were recorded using YSI 556 Multi-Probe Meter. The correlation of physical parameters with fish parasites showed that only protozoan cyst (DO: r = 0.806, P < 0.05) and Acanthocephalus sp.(DO: r= 0.959, P < 0.05) showed a significant positive correlation with DO. Unidentified protozoan cysts, Acanthocephalus sp. and Dactylogyrus sp. 2 D. hamacopulus, Dactylogyrus sp. 3, and Paradiplozoon barbi. Acanthosentis sp., Ergasilus sp., D. dolichoirri, unidentified adult trematode, unidentified larval trematode, Lamproglena sp. 2, Gyrodactylus sp., unidentified nematode, and unidentified copepod showed a relationship with pH, DO, and temperature, while others do not. Majority of the parasites weakly correlated with physical water parameters due to the irregular measurement of water quality of the reservoirs during the study. In conclusion, the value of pH, DO and temperature recorded in this study is suitable for fish survival. This research revealed that Unidentified protozoan cyst and Acanthocephalus sp. showed a significant positive correlation with DO values of the reservoirs along Perak River.

Keywords: Dissolved Oxygen, fish parasites, pH, River Perak Reservoirs temperature

INTRODUCTION

The abundance and composition of fish parasites are directly affected by the physico-chemical parameters of the aquatic habitat (Gilbert and Avenant-Oldewage, 2017). Besides, human activities are increasingly affecting water quality. The physico-chemical parameters that influence parasitic infection are Dissolved Oxygen (DO), pH, ammonia, conductivity, turbidity and temperature (Saha *et al*., 2013). Diffusion from the air and photosynthetic aquatic plants add Dissolved Oxygen (DO) to the water body which is the oxygen source for fish and other aquatic organisms (Boyd, *et al*., 2018). When the Dissolved Oxygen (DO) concentration is less than 2-3 mg/L as a result of eutrophication which leads to depletion of Dissolved Oxygen (DO) (hypoxia), most aquatic organisms cannot survive, 5 mg/L is the minimum concentration required for the survival of aquatic organisms (Ficke *et al.*, 2007). Fishes infected with a high number of parasites perish first in a moderate to low oxygen concentration. According to Budria (2017), fishes with high prevalence rate of parasitic infections do not live longer due to the substantial reduction in the respiratory surface of the gill which interferes with the respiration process for energy production, catabolism and anabolism as a result of parasitic infection on the gills.

A range between 6 and 9 is the best pH value for freshwater fish growth and survival (Stone and Thormforde, 2003). According to Lewis (2016), pH of the aquatic habitat affects host-parasite interactions in fish. Monogeneans and digeneans prevalence were high in less acidic aquatic habitat than in more acidic location, since the free-swimming larvae of monogeneans and the first molluscan intermediate hosts of digenetic trematodes are rare to absent in water of low pH owing to their acid-sensitivity (Lewis, 2016). While copepods and acanthocephalans are not affected by high acidity, since the free-swimming copepod larva and acanthor larva can survive in water of low pH. The infective larvae stages are highly resistant to stress caused by acidification. A low prevalence rate of *Paraergasilus rylovi* (copepod) at pH below 5 - 6 has been reported since the pH for reproduction and growth in the aquatic habitat is not optimal for the parasites (Saarinen and Taskinen, 2004).

Parasitic distribution, growth, transmission, host hormone cycle and survival are significantly influenced by the temperature of the water (Hakalahi *et al*., 2006). The effect of temperature on fish parasite is dynamic, increased temperature results in a higher prevalence of some parasitic diseases by enhancing parasites metabolism, giving to a high number of parasites transmission stages being produced resulting in a high prevalence of parasitic disease, while for other parasites there is no effect or negative effect if their optimal temperature for transmission and growth is exceeded (Karvonen *et al.,* 2010). The immune system of freshwater fish is affected by temperature which is the most vital physiological protection against parasitism (Hernández and Tort, 2003). This study was initiated due to the lack of information available related to influence of abiotic factors in freshwater fish parasitism in Temengor, Bersia and Chenderoh reservoirs.

MATERIALS AND METHODS

Fish samples were obtained from Temengor reservoir located between latitude 5.41^o North and Longitude 101.22^o East, Bersia reservoir Located between latitude 5.41^o North and Longitude 101.22^o East and Chenderoh reservoir located between latitude 5.02^o North and longitude 100.97^o East (Figure 1). These reservoirs are all located along the Perak River. The Perak River is 427 km long and the second-longest river in Peninsular Malaysia (Hashim *et al.*, 2012). Live fish were randomly sampled from fishermen from the reservoirs for 23 months. Battery power aerated cooler with the reservoir water was used to transport the live fishes to Universiti Sains Malaysia laboratory and identified using key prepared by Rainboth (1996) and Froese and Pauly (2019). Smeared were made from the skin scrapping and observed under the microscope for ectoparasites. Fish were dissected and examined for internal parasites using standard parasitological techniques. Parasites were identified after Anderson *et al.*,(1980), Gussev (1985), Hoffman (2019), Kabata (1979, 1985) Paperna (1996), and Yamaguti (1958, 1961a, 1961b).

Three replicates of *in-situ* water physicochemical parameters were taken at each sampling period from the reservoirs. (Table 1). Dissolved oxygen (DO) concentration (mg/L), temperature (^{o}C), and pH of the reservoirs were determined using YSI 556 Multi-Probe Meter (YSI Incorporated, Yellow Spring, Ohio, USA), which was calibrated according to manufacturer specifications during the study periods.

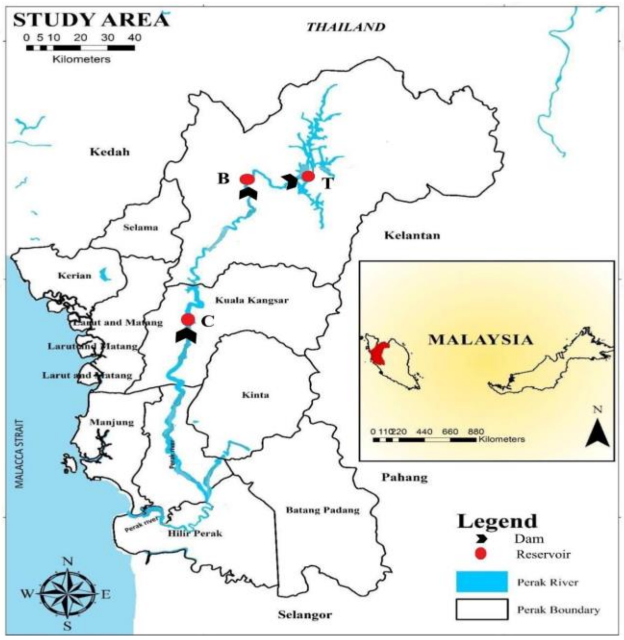

Figure 1: Location of Temengor (**T**), Bersia (**B**), Chenderoh (**C**) Reservoirs along the Perak River. Adapted from Salam *et al*., (2019).

Table 1. Study location, season and date of sampling periods

Study location	Season	Sampling date
Chenderoh	Non monsoon	08/04/2017
Temengor	Non monsoon	10/06/2017
Bersia	Non monsoon	10/06/2017
Temengor	Non monsoon	13/08/2017
Temengor	Monsoon	04/11/2017
Bersia	Monsoon	04/11/2017
Chenderoh	Monsoon	21/01/2019
Chenderoh	Non monsoon	30/03/2019

Pearson's correlation analysis was used to compare the impact of abiotic factors in the reservoirs with fish parasitic presence using IBM SPSS Statistics software version 24 (New York, USA). The Interpretation of the correlation coefficient of abiotic factors and parasites prevalence is according to Schober *et al.* (2018) as shown in Table 2.

Table 2: Interpretation of the correlation coefficient

Value of coefficient r (positive or negative)	Interpretation
0.00- 0.19	A very weak correlation
0.20- 0.39	A weak correlation
0.40- 0.69	A modest correlation
0.70 – 0.89	A strong correlation
0.90 – 1.00	A very strong correlation

Canonical Correspondence Analysis (multivariate CCA) was used to investigate the relationship between parasites and physico-chemical parameters using PAST software version 4.03 (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

Table 3 showed the variations in temperature, DO and pH recorded during the study period. Changes in anthropogenic activities and weather conditions caused variations recorded for DO, pH, and temperature in Temengor, Bersia, and Chenderoh reservoirs during the study period (Salam *et al*., 2019). In this study, the high value of DO was recorded. According to Shen *et al.* (2013) and Chennakrishnan *et al.* (2008), this may be because of run-off from the monsoon season, oxygen production through photosynthesis by phytoplankton and aeration from the atmosphere. The satisfactory amount of DO needed by aquatic organisms was between 5.3 mg/L and 8.0 mg/L.

The value of pH recorded in this study is within the range (6.6 – 8-5) required for biological activities in reservoirs (Ibrahim *et al.,* 2009). The variation of pH within the reservoirs may be caused by changes in temperature, water body exposure, biological activities, and decaying death organism (Yerima *et al.*, 2017). Similarly, the variation of temperature within the reservoirs is influenced by weather conditions and periods when the temperature is measure (Muhammed *et al.*, 2018).

Table 3: Water physico-chemical parameters recorded during the study period. in Temengor, Bersia and Chenderoh reservoirs along the Perak River.

Sampling date	Season	Reservoirs	Dissolved oxygen (mg/L)	pH	Temp.(°C)
08/04/2017	Non monsoon	Chenderoh	5.47 ± 0.69	7.51 ± 0.45	29.84 ± 1.00
10/06/2017	Non monsoon	Temengor	7.93 ± 0.56	7.60 ± 0.51	31.73 ± 0.99
10/06/2017	Non monsoon	Bersia	5.65 ± 0.87	6.59 ± 0.89	29.14 ± 1.21
13/08/2017	Non monsoon	Temengor	6.15 ± 0.79	7.59 ± 0.39	29.00 ± 1.39.
04/11/2017	Monsoon	Temengor	7.02 ± 0.93	6.94 ±0.90	29.83 ±1.18
04/11/2017	Monsoon	Bersia	5.93 ± 0.89	6.31 ± 0.91	29.56 ± 1.56
21/01/2019	Monsoon	Chenderoh	5.50 ± 0.69	6.94 ± 0.78	28.10 ± 1.11
30/03/2019	Non monsoon	Chenderoh	5.36 ± 0.93	7.20 ± 0.42	31.30 ± 1.23
		Mean ±SD	6.13 ± 0.90	7.09 ± 0.48	29.81 ±1.20
		Range	5.36 – 7.93	6.31- 7.60	28.10 – 31.73

Values represent Mean ±SD

From the correlation matrix between parasites prevalence and water physicochemical parameters of the reservoirs (Table 4). Unidentified protozoan cysts **(**DO: r = 0.806, P < 0.05) and *Acanthocephalus* sp**. (**DO: r= 0.959, P < 0.05) shows a significant positive strong correlation with DO, while *Balantidium* sp., *Camallanu*s sp. and *Procamallanus* sp. negatively moderately correlated among the 32 species of parasites. In a similar case in Kenyir Lake, Terengganu, Malaysia, Modu (2013) reported the prevalence of freshwater fish parasite shows a significant perfect positive (P < 0.05) correlation with DO. According to Barson *et al.* (2008), high concentration of DO increases the prevalence and proliferation of the parasite. These results differ from Alsarakibi *et al.* (2012), which showed that *Argulus japonicus* prevalence was positively related to DO, but the prevalence rate was very high at a low amount of DO since *A. japonicus* feed on the host blood and obtain oxygen from haemoglobin in the host's blood tissues.

The correlation of parasites with pH in this study indicates that the parasite's prevalence rate reduces with increasing pH. *Dactylogyrus dolichoirri*, *Lamproglena* sp. 2 and unidentified larva trematode shows a significant negative modest correlation with pH. While *Dactylogyrus* sp. 1 shows a significant positive modest correlation with pH in the study. According to Saha *et al.* (2013), fish parasites prevalence and reproduction is enhanced by lower pH of the freshwater habitat.

Table 4: Pearson correlation matrix between the prevalence of 32 species of parasites with dissolved oxygen, pH and temperature in Temengor, Bersia and Chenderoh reservoirs along the Perak River.

Parasite species	**Correlation (*r*) between prevalence of parasite and DO (mg/L)**	**Correlation (*r*) between prevalence of parasite and pH**	**Correlation (*r*) between prevalence of parasite and Temp.(°C)**
Balantidium sp.	-0.510*	-0.127	0.253
Unidentified Protozoa cyst	0.806 *	0.435	0.648 *
Henneguya sp.	-0.254	0.112	-0.682*
Myxobulus sp.	-0.423	0.159	-0.404
Dactylogyrus sp. 1	-0.266	0.559*	-0.137
D. tapienensis	-0.293	0.359	0.009
Dactylogyrus sp. 2	0.297	0.260	-0.172
Dactylogyrus sp. 3	-0.009	0.283	-0.296
D. hamacopulus	-0.053	0.378	-0.469
D. lampam	-0.470	0.019	0.128
D. dolichoirri	-0.111	-0.523*	-0.312
Gyrodactylus sp.	0.399	-0.122	0.006
C. sundanensis	-0.250	0.126	-0.682*
C. sclerosus	-0.321	0.203	-0.634*
S. longicornis	-0.280	-0.122	-0.579*
P. barbi	-0.069	0.086	-0.400
O. malayea	-0.213	0.418	-0.227
Unidentified larva trematode	-0.229	-0.672*	-0.386
Unidentified adult trematode	0.043	-0.464	-0.213
Bothriocephalus sp.	-0.342	0.097	0.503
Camallanus sp.	-0.612*	-0.192	-0.008
Procamallanus sp.	-0.502*	-0.279	-0.290
Cucullanus sp.	-0.213	-0.418	-0.227
Unidentified nematode	0.399	-0.122	0.006
Acanthocephalus sp.	0.959*	0.338	0.604*
Acanthogyrus sp.	-0.405	-0.083	-0.376
Acanthosentis sp.	-0.128	-0.461	-0.586*
Lamproglena sp. 1	0.080	0.011	-0.153
Lamproglena sp. 2	0.238	-0.594*	-0.061
Lamproglena sp. 3	0.399	-0.122	0.006
Ergasilus sp.	0.399	-0.122	0.006
Unidentified copepod	0.399	-0.122	0.006

*Correlation is significant at 0.05 level (2-tailed). Absence of (*) indicate weaker correlation at 0.05 level (2-tailed).

Similarly, water temperature in the present study moderately significantly correlates with some parasites. *Henneguya* sp., *C. sundanensis*, *C. sclerosis*, *S. longicorris* and *Acanthosensis* sp. shows a significant negative moderate correlation with water temperature, while unidentified protozoan cyst and *Acanthocephalus* sp. shows a significant positive moderate correlation with water temperature in this present study. According to Eissa, (2006), parasites prevalence is high especially in low water temperature. While Franke *et al,* (2017), reported that the rate of parasitic prevalence increases by rise in temperature. These differences are attributed to the effect of temperature on parasites which may be positive by accelerating the life cycle of the parasite and increase parasitism or negative as it may be fatal for certain parasite species leading to low prevalence (Karvonen *et al*., 2010).

Majority of the fish parasites weakly correlate negatively or positively with pH, DO and temperature as indicated in the results. According to Gomesa *et al.* (2017), water quality parameters can change over a short time and therefore recommends regular monitoring of water quality parameters which can eventually is influence correlationship of parasites and physicochemical parameters.

Table 5 shows the Pearson correlation matrix between the prevalence of parasites in monsoon and non-monsoon seasons with DO, pH, and water temperature in Temengor, Bersia and Chenderoh reservoirs along the Perak River. This study recorded some changes in seasonal prevalence between monsoon and non-monsoon seasons, thus indicating that water quality parameters could have a direct association with parasites existence. The prevalence of parasites negatively correlated with DO and water temperature in the monsoon season. In the non-monsoon season, temperature showed a moderate negative significant correlation with seasonal prevalence of parasites. According to Wali *et al*. (2016), parasites infection rate is high during the monsoon season and low during the non-monsoon season, this showed seasonal alteration associated with environmental changes.

According to Gomesa *et al.* (2017), physicochemical parameters can varied over a short period of time and recommends regular sampling of physicochemical parameters and which can eventually is influence correlationship of parasites and physicochemical parameters.

Table 5. Pearson Correlation between seasonal prevalence and water physico-chemical parameters.

Seasonal prevalence of parasites	Dissolved oxygen (mg/L)	pH	Temperature (°C)
Non-monsoon prevalence	-0.288	-0.133	-0.577*
Monsoon prevalence	-0.891*	0.224	-0.988*

*Correlation is significant at 0.05 level (2-tailed). Absence of (*) indicate weaker correlation at 0.05 level (2-tailed).

Figure 2 shows the Canonical Correspondence Analysis biplot (multivariate CCA) between the prevalence of the 32 species of parasites with dissolved oxygen, pH, and water temperature in Temengor, Bersia and Chenderoh reservoirs along the Perak River. Canonical correspondence analysis biplot was constructed to provide a visual assessment of the relationship between parasites and water quality parameters of River Perak reservoirs.

Therefore, it gives more detailed characteristics of the interactions between ecosystem factors and fish parasites. Water quality parameters have been linked to the prevalence of some parasites, DO measure to unidentified protozoan cysts, *Acanthocephalus* sp. and *Dactylogyrus* sp. 2 as this parameter enhances infection and reproduction success of these parasites 147 (Barson *et al*, 2008). Similarly, pH level also influences the prevalence of *D. hamacopulus, Dactylogyrus* sp. 3, and *P. barbi*. According to Saha *et al.* (2013), parasite prevalence is high in less acidic freshwater habitats.

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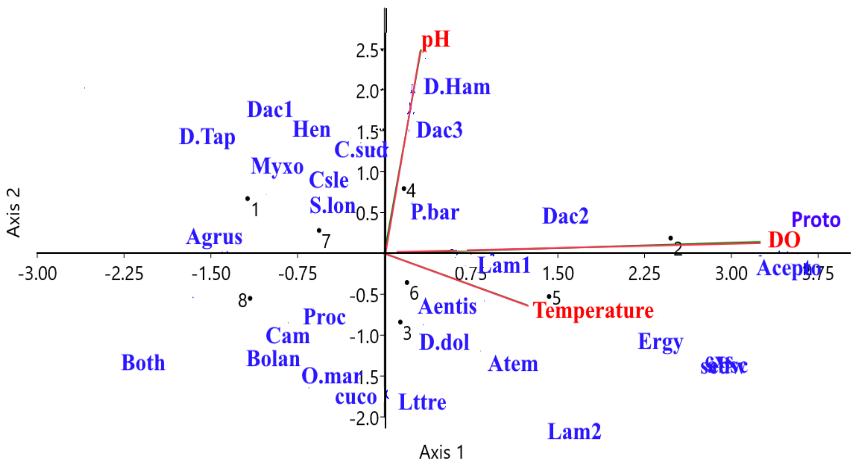

Figure 2: Canonical Corresponding Analysis (multivariate CCA) showing the distribution of fish parasites in relation to pH, Dissolved oxygen (DO mg/L) and Water temperature (°C) in reservoir along Perak reservoirs during the study period. Abbreviation for parasites names: Bolan, *Balantidium* sp.; Proto, Unidentified Protozoan cyst; Hen,*Henneguya* sp; Myxo, *Myxobulus* sp.; Dac1, *Dactylogyrus* sp.1; Dac2, *Dactylogyrus* sp.2; Dac3, *Dactylogyrus* sp.3; D.Ham, *D. hamacopulus;* D.Lam, *D. lampam;* D.dol, *D. dolichoirri*.; C.sud, *C. sundanensis;* C.sle, *C. sclerosus;* P.bar, *P. barbi;* O.mal, *O. malayea;* Ltrem, Unidentified larva trematode*;* Atrem, Unidentified adult trematode*;* Both, *Bothriocephalus* sp**.;** Cam**,** *Camallanus* sp.; Proc, *Procamallanus* sp*.;* cuco, *Cucullanus* sp.; Aceph, *Acanthocephalus* sp.; Agyrus, *Acanthogyrus* sp; Aentis, *Acanthosentis* sp.; Lam1, *Lamproglena* sp.1; Lam2, *Lamproglena* sp.2; Ergy, *Ergasilus* sp. Five parasites clustered at the same point are Gyro, *Gyrodactylus* sp.*; ;* Cop, Unidentified copepod; Nem, Unidentified nematode, Lam2, *Lamproglena* sp.2 and Lam3, *Lamproglena* sp. 3.

Water temperature was associated with the prevalence level of *Acanthosentis* sp., *Ergasilus* sp., *D. dolichoirri*, unidentified adult trematode, unidentified larval trematode, *Lamproglena* sp. 2, *Gyrodactylus* sp., unidentified nematode, and unidentified copepod as this variable increases parasitic growth and reproduction (Hakalali *et al.*, 2006).

In the present study some parasites did not show any relationship to water quality. According to Gomesa *et al*. (2017), water quality parameters are not stable and therefore need regular measurement. In this study, water quality parameters were measured irregularly. Therefore, this might be the reason why parasites did not show any relationship with pH, dissolved oxygen, and water temperature in the canonical correspondence analysis biplot results.

CONCLUSION AND RECOMMENDATION

Abiotic and biotic factors could strongly affect fish parasite communities in the reservoirs, and long-term study should be conducted on the influence of physio-chemical parameters and biotic factors (intermediate host) on the fish parasite communities. Such studies might be useful for the rational management of the fisheries of the reservoirs along the Perak River. Thirty-two species of freshwater fish parasites were correlated with pH, temperature, and DO. Unidentified protozoan cyst and *Acanthocephalus* sp. show a significant correlation with DO. Majority of the parasites weakly correlate with the physico-chemical parameters because of irregular measurement of water quality during the study period. Some fish parasites showed positive or negative relationship with pH, DO and temperature, while others do not.

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