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Freshwater Fish Parasites in Temengor, Chenderoh and Bersia Reservoirs, Perak, Malaysia

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ABSTRACT
Parasitic infection causes decrease in wild fish population and financial losses in culture fish. Fundamental biological variables like fish parasite composition baseline data and fish hosts-parasites interactions on fish parasites are needed in establishing a monitoring and management system of these freshwater fishes to mitigate the possible adverse effect of these parasitic diseases. There is no information about protozoa and myxozoa parasitic fauna of the fishes in River Perak reservoirs. The prevalence of freshwater fish parasites in Temengor, Bersia and Chenderoh Reservoirs, Perak was investigated for 23 months for fish parasites using standard parasitological techniques. A total of 32 species of parasites detected belonging to 8 major taxa: 2 species of protozoans, 2 species of myxozoa, 12 species of monogeneans, 3 species of digeneans, 4 species of nematodes, 3 species of acanthocephalans and 5 species of copepods. *Balantidium* sp., *Henneguya* sp., and *Myxobulus* sp. were newly recorded parasites species in the reservoirs. The prevalence of parasitic infection in the reservoirs were 51 % in Temengor, Bersia 61% and Chenderoh 65 %. The parasite diversity of the fish parasites per reservoirs were moderate. The parasite with the highest prevalence rate was recorded in *Paradiplozoon barbi* (17.39 %). Eighteen fish species were infected with parasites out of the twenty-five species sampled from the reservoirs. The fish family Cyprinidae was the dominant and most parasitized fish family in the reservoirs.

Keywords: Fish Parasites; Prevalence; River Perak; Reservoirs

INTRODUCTION
Perak River is the second longest river in Peninsular Malaysia and has four reservoirs along its length. These reservoirs were constructed in different periods as a result of building dams along the river (Hashim *et al*., 2012). Freshwater fish in the reservoirs can be predisposed to diseases, especially in stressful conditions. The presence of pathogenic organisms in the reservoirs also cause diseases in freshwater fish (Arringnom, 1998). Bad water quality exposes fishes in the reservoirs to be subjected to many parasitic diseases. Parasitic infection causes an increasing amount of death in the fish population (Yadav, 2000). The amount of food available to human beings around the world is reduced due to parasitic diseases of fish (and livestock). According to Roberts (2012), more than half of the total biodiversity of living organisms on the globe comprises of parasites. Species parasitic on freshwater fish is measured in thousands and many more fish parasites remain to be discovered and described (Roberts, 2012).
Fish parasites have various means to infect the fish host which include depriving the fish host enough food or vital nutrient like vitamins, localizing, and outnumbering their population in vital organs of the fish host which leads to diseases (Mehlhorn, 2001). In Malaysia, information of parasites of standing water like reservoirs are few (Szekely *et al*., 2009).

There were no documented reports on the prevalence of protozoans and myxozoa parasitic fauna in Temengor, Chenderoh and Bersia Reservoirs. Most studies were done on parasitic helminths and crustaceans (Bu and Leong, 1997, 1999; Lim *et al*., 2016). Fundamental biological variables like fish parasite composition baseline data and fish hosts-parasites interactions on fish parasites are needed in establishing a monitoring and management system of these freshwater fishes to mitigate the possible adverse effect of these parasitic diseases**.** Hence, the need for research on freshwater fish parasites in these reservoirs is therefore necessary. The research was conducted to identify the parasites from the freshwater fishes in Temengor, Bersia and Chenderoh reservoirs in Perak River.

MATERIALS AND METHODS

Experimental Site

Temengor, Bersia and Chenderoh reservoirs are located on the Perak River (Fig 1). Temengor Reservoir has an area of 152 Km^2, located between latitude 5.55^o North and longitude 101.34^o East. Bersia Reservoir is Located latitude 5.41^o North and Longitude 101.22^o East with an area 5.7 Km^2 and Chenderoh Reservoir is located between latitude 5.02^o North and longitude 100.97^o East with an area of 21Km^2 (Hashim *et al*., 2012).

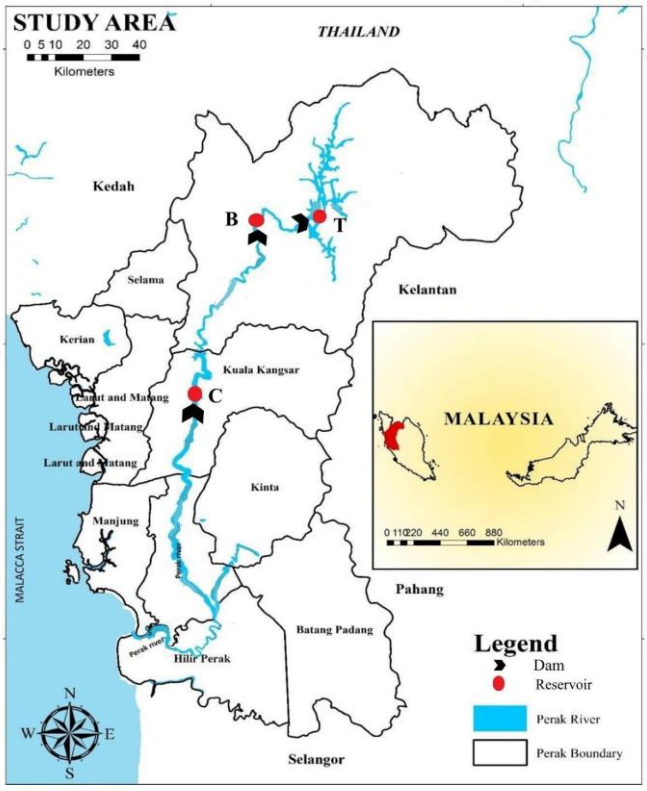

Figure 1: Map showing the study area along River Perak. Temengor Reservoir (T), Bersia Reservoir (B) Chenderoh Reservoir (C). Source: Salam *et al*., (2019).

Sample Collection

Live fish were randomly sampled from fishermen from Temengor, Bersia and Chenderoh reservoirs for 23 months. Battery power aerated cooler with the reservoir water was used to transport the live fishes to USM 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 (1967), Kabata (1979, 1985) and Paperna (1996).

Statistical analysis

The prevalence of parasites and mean intensity of infection of fish in Temengor, Bersia, and Chenderoh Reservoirs were calculated according to Bush *et al*., (2001). Shannon Wiener's diversity index was used to calculate the diversity of parasites in the different reservoirs (Magurran, 1988). The formula adapted were as follows:

$$\text{Prevalence} = \frac{\text{Number of fish infected}}{\text{Total number of fish}} \times 100\ \%$$

$$\text{Mean intensity} = \frac{\text{Number of parasites found}}{\text{Total number of infected fish}}$$

(Bush *et al.*, 2001).

9Shannon Wiener's diversity index was used to calculate the diversity of parasites in the different reservoirs as define in Magurran, (1988).

Shannon Wiener's diversity index (H') = $-\sum$ pi log pi

Where H' is diversity index, $\sum$ is summation, log is logarithm, pi is the relative abundance of species "i" in the community (Magurran, 1988).

RESULTS

Among the total of 276 live fish were examined for parasites 65 % were obtained from Chenderoh (25 % fish), Bersia (109 fish), and Temengor (98 fish) for 23 months. One hundred and sixty-one (161) of these fish (58 %) were infected with parasites. The prevalence of parasitic infection was high in Chenderoh Reservoir (65 %) while Temengor Reservoir had the lowest prevalence (52 %). The parasite diversity in the reservoirs was moderate (H= 2.14) (Table 1).

Table 1: Sampled reservoirs, the total number of fish sampled, prevalence (%) of infected fish, the total number of parasites species found per reservoir(S) and parasite diversity per reservoir (H).

Reservoir	Number of fish sampled	Percentage of fish sampled	Number of infected fish	Prevalence (%)	S	H
Chenderoh	69	25.00	45	65	18	1.92
Bersia	109	39.49	66	61	18	1.97
Temengor	98	35.51	50	51	21	2.47
Total	276	100	161	58	33	2.14

Parasites diversity (H) Interpretation: 1=High diversity, 2=Moderate diversity, 3=Low diversity

Table 2 shows the total fish species sampled, the number of infected fish and, prevalence of infected fish. Twenty-five species of fish sampled belong to seven families. Majority of the fish sampled belong to the Cyprinidae, comprising of 17 fish and out of it fifteen were infected with parasites. *Tor tambra, Rasbora species, Puntius binotatus,* and *Puntius brevis* had the highest prevalence (100%) in the Cyprinidae. Cichlidae comprised of two fish species, with *Oreochromis niloticus* recording highest prevalence of 90 %.

Table 2**:** Fish species, total number sampled, number of fish infected and prevalence of infected fish in Temengor, Bersia, and Chenderoh reservoirs.

Fish species/family	Number of fish sampled	Number of infected fish	Prevalence (%)
Cyprinidae			
Osteochilus vittatus	50	21	42
Cyclocheilichthys apogon	21	15	71.4
Cyclocheilichthys species	3	1	33.3
Labiobarbus leptochelus	34	16	47.1
Osteochilus melanopleurus	3	1	33.3
Hampala macrolepidota	37	10	27
Cyclocheilichthys armatus	5	0	0
Labiobarbus festivus	2	0	0
Osteochilu species	4	3	75
Poropunthus deaurantus	6	3	50
Barbonymus schwanefeldii	38	33	86.8
Puntius brevis	1	1	100
Putioplites bulu	26	25	92.3
Mystacoleucus marginatus	19	14	73.7
Puntius binotatus	4	4	100
Rasbora spp.	1	1	100
Tor tambra	1	1	100
Cilchlidae			
O*reochromis niloticus*	10	9	90
Cichla ocellaris	1	0	0
Nandidae			
Pritolepsi grootii	1	0	0
Pritolepsi fasciata	3	0	0
Notopteridae			
Notopterus notopterus	3	0	0
Channidae			
Channa striata	1	0	0
Osphroneminae			
Osphronemus goramy	1	0	0
Bagridae			
Hemibagrus nemurus	1	1	100

The *Notopterus notopterus* species (Notopteridae) and Hemibagus (Bagridae) all recorded the highest prevalence (100 %). The freshwater fish parasites isolated from this research were two protozoans (*Balantidium* sp. and unidentified protozoa cyst in the intestine and gill, respectively), two myxozoa (*Henneguya* sp. and *Myxobulus* sp. from the gills), twelve monogeneans (three *Dactylogyrus* spp., *D. tapiensis, D. hamacopulus, D. lampam, D. dolichoirri, Gyrodactylus* sp. , *Cichlidogyrus sclerosus, Scutogyrus longicornis, Cornudiscoides sundanensis* and *Paradiplozoon barbi* from the gills), three digeneans (*Osteochilotrema malayea*, unidentified adult trematode from the intestine and unidentified larva trematode from the gill cover), one cestode (*Bothriocephalus* sp. from the intestine), four nematodes (*Camallanu*s sp., *Procamallanus* sp., *Cucullanus* sp. from the intestine and unidentified nematode from the stomach), three acanthocephalans (*Acanthocephala* sp., *Acanthogyru*s sp. and *Acanthosentis* sp. from the intestine), and five crustaceans (three *Lamproglena* spp., *Ergasilus* sp. and unidentified copepod from the gills). The prevalence, mean intensity, location of identified fish parasites in Chenderoh, Bersia and Temengor Reservoirs is summarized in Table 3. The highest prevalence of parasitic infection was recorded on *P. barbi* (17.39 %) followed by *Dactylogyrus tapienensis* (7.97 %) and *Dactylogyrus hamacopulus* (7.61 %). *Myxobulus* sp., *Henneguya* sp. (Myxobulus) and *Balantidium* sp. (protozoa) are not previously reported among fish parasites found in reservoirs. The Maximum mean intensity of parasites was found on *Bolantidium* sp. (77.71 %).

Table **3:** Prevalence (P), mean intensity of parasite identified in Temengor, Bersia, and Chenderoh Reservoirs, Perak.

Reservoir infected	Parasite species/ Taxonomic group	Number of infected fish	Fish host with infection	P (%)	Mean Intensity
Bersia, Temengor, Chenderoh	Protozoan *Bolantidium* sp.*	14	*Labiobarbus leptochelus, Osteochilus vittatus , Barbonymus schwanefeldii*	5.07	77.71±0.32
Temengor	Unidentified Protozoan cyst	3	*Osteochilus vittatus* ,	1.08	2.6 ± 0.002
Bersia, Chenderoh	Myxozoa *Henneguya* sp.*	3	*Putioplites bulu*	1.09	33 ± 0.009
Bersia, Chenderoh	*Myxobulus* sp.*	5	*Putioplites bulu, Barbonymus schwanefeldii*	1.81	27.4±0.04
Chenderoh, Temengor	Monogenean *Dactylogyrus* sp. 1	3	*Hampala macrolepidota, Putioplites bulu*	1.09	11 ± 0.01
Chenderoh, Temengor	*Dactylogyrus* tapienensis	2	*Puntius brevis, Puntius binotatus*	0.72	10 ± 0.005
Bersia, Temengor	*Dactylogyrus* sp. 2	8	*Osteochilus vittatus Osteochilus* species	2.89	3.75±0.008
Bersia, Temengor	*Dactylogyrus* sp. 3	5	Mystacoleucus marginatus	1.81	9.8 ± 0.01
Chenderoh, Temengor, Bersia	*Dactylogyrus hamacopulus*	21	*Putioplites bulu*	2.61	3.04 ± 0.08
Chenderoh	*Dactylogyrus lampam*	22	*Barbonymus schwanefeldii*	7.97	11.45±0.07
Bersia, Temengor	*Dactylogyrus dolichoirri*	20	*Cyclocheilichthys* species, *Cyclocheilichthys apogon, Labiobarbus leptochelus*	7.25	15 ± 0.08
Temengor	*Gyrodactylus* sp.	1	*Putioplites bulu*	0.36	3 ± 0.0008

Table 3 continue

Chenderoh	*Cornudiscoides sundanensis.*	4	*Hemibagrus nemurus*	1.44	19 ± 0.02
Chenderoh	*Cichlidogyrus sclerosus*	5	O*reochromis Niloticus*	1.81	36.6 ± 0.05
Chenderoh	*Scutogyrus longicornis*	5	O*reochromis niloticus*	1.81	33.4 ± 0.04
Bersia, Temengor, Chenderoh	*Paradiplozoon barbi*	48	*Cyclocheilichthys apogon, Labiobarbus leptochelus, Puntius binotatus, Osteochilus vittatus, Tor tambra, Poropunthus deaurantus,* Mystacoleucus marginatus, *Hampala macrolepidota, Barbonymus schwanefeldii*	17.39	3.18 ± 0.04
Bersia	Digenean *Osteochilotrema malayea*	2	*Osteochilus vittatus,* *Osteochilus* species	0.72	1.5 ± 0.008
Bersia, Chenderoh	Digenean Unidentified larva Trematode	3	*Labiobarbus leptochelus, Barbonymus schwanefeldii*	1.09	3.6 ± 0.003
Bersia	Unidentified adult Trematode	11	*Osteochilus vittatus, Labiobarbus leptochelus Rasbora s* spp.,	3.98	2.63±0.0086
Chenderoh	Cestoda *Bothriocephalus* sp.	1	*Barbonymus schwanefeldii*	0.36	1 ± 0.00029
Chenderoh, Bersia	Nematoda *Camallanus* sp.	11	*Hampala macrolepidota, Barbonymus schwanefeldii, Cyclocheilichthys apogon,*	3.98	3.9 ± 0.01
Chenderoh, Bersia	*Procamallanus sp.*	22	*Barbonymus schwanefeldii,*	7.97	4.4 ± 0.02
Bersia	*Cucullanus* sp.	2	*Hampala macrolepidota,*	0.72	5 ± 0.003
Temengor	Unidentified nematode	5	*Putioplites bulu, Cyclocheilichthys apogon,*	1.81	16.2 ± 0.02

Table 3 Continue…

Temengor	Acanthocephala *Acanthocephalus* sp.	3	*Osteochilus vittatus,*	1.09	4.7 ± 0.004
Chenderoh	*Acanthogyrus* sp.	8	*Osteochilus vittatus, Barbonymus schwanefeldii,*	2.89	2.62 ± 0.006
Bersia, Temengor, Chenderoh	*Acanthosentis* sp.	7	*Osteochilus vittatus, Barbonymus schwanefeldii,*	2.53	1.28 ± 0.002
Bersia, Temengor	Crustacean *Lamproglena* sp. 1	6	*Osteochilus vittatus, Putioplites bulu, Labiobarbus leptochelus*	2.17	1.16 ± 0.002
Bersia, Temengor	*Lamproglena* sp. 2	3	*Labiobarbus leptochelus*	1.09	1.33 ± 0.001
Temengor	*Lamproglena* sp. 3	1	*Putioplites bulu*	0.36	1 ± 0.0003
Temengor	*Ergasilus* sp.	1	Mystacoleucus marginatus,	0.36	1 ± 0.003
Temengor, Chenderoh	Unidentified copepod	4	*Osteochilus vittatus,* O*reochromis niloticus*	1.44	1 ± 0.001

*designate new recorded parasites species

High prevalence of fish parasites were obtained from *Barbonymus Schwanefeldii* followed by *Osteochilus vittatus*, *Cyclocheilichthys apogon* and *Labiobarbus leptochelus.* The 15 infected fish hosts species out of 17 infected fish hosts analysed belong to the family Cyprinidae with *B. Schwanefeldii* recorded the highest number fish parasites.

DISCUSSION

The diversity of parasites in the reservoirs were moderate (H=2.4). A very important factor influencing parasites' prevalence and diversities in the reservoirs is the age of the reservoirs (Song & Proctor, 2020) and differences in biotic and abiotic factors among the reservoirs (Wali *et al*., 2016).. In this research, Chenderoh Reservoir has the highest prevalence of parasites and the oldest among the reservoirs (seventy years old) because it is more established in terms of accommodating plants and animals, therefore prone to organic pollution , while Temengor and Bersia Reservoirs had lower prevalence, this is in agreement with the result of Bu and Leong (1997), which was also highest in Chenderoh Reservoir.

Majorities of the fish samples belong to the Cyprinidae, comprising of seventeen fish species. Fifteen fish species of the cyprinids were infected with parasites. *Tor tambra, Rasbora sp., Puntius binotatus and Puntius brevis* had highest prevalence (100 %) in the Cyprinidae. *Oreochromis niloticus* (Cichlidae) had a prevalence of 90 % and *Hemibagrus nemurus* (Bagridae) also recorded 100 % prevalence. Most of the parasitic infections of the fishes in the reservoirs were dominated by monogeneans. According to Bu and Leong (1997), majority of the freshwater fish in the reservoirs were infected by parasitic monogeneans.

Three new recorded parasites species were reported for the first time in the reservoirs, they are *Balantidium* sp. isolated from the intestine of *L. leptochelus, O. vittatus* and *B. schwanefeldii* from Bersia, Temengor and Chenderoh Reservoirs., *Myxobulus* sp. isolated from the gills of *P. bulu* and *B. schwanefeldii* from Bersia and Chenderoh Reservoirs and *Henneguya* sp. obtained from the gills of *P. bulu* from Bersia and Chenderoh Reservoirs. Previous studies focusing on fish parasites in reservoirs along the Perak River were on helminths and crustaceans (Bu and Leong 1977, 1999; Shen-yin *et al*., 2016).

Most of the parasite species identified from the reservoirs were dominated by monogeneans. The most abundant parasites of freshwater fish are monogeneans parasites (Ivona, 2004). According to Luque and Poulin, (2007) and Bellay *et al.*, (2015), monogeneans are fish parasites that are dominant in the fish parasites community in all tropical reservoirs.

The prevalence of digenean recorded from this study was low. Rahman and Bakri (2008) reported the prevalence value of 70 % on trematodes in Kedah, Peninsular Malaysia. This may be a result of differences in biotic and abiotic factors in the aquatic habitat of the study area (Rahman and Bakri, 2008). Only one species of cestode with very low prevalence was found in this study. This is as a result of a low number of intermediate hosts for the cestode (Leong *et al.* 1987). Four species of nematodes and three species of acanthocephalans were recovered from this study with low prevalence and intensity. The prevalence of these parasites was influenced by the feeding behaviour and diet of the fish host (Beevi and Radhakrishnan, 2012). Low prevalence and mean intensity of crustaceans were observed in this study. The parasitic infestation of external parasitic crustaceans is affected by various abiotic factors in the reservoirs. Parasitism of crustaceans is via contact on skin and gills. Environmental factors have a strong influence on the prevalence rate of parasitic crustaceans and when it is unsuitable for the development of crustaceans it results to low prevalence rate (Vasconcelos and Tavares-Dias, 2016). Changes in physio-chemical parameters of the aquatic habitat may affect the prevalence rate of the parasite by possibly hindering the survival of certain stages of the life cycle of parasitic crustaceans (Tavares-Dias *et al.*, 2015).

The 32 species of fish parasites form the parasitic community of the 17 infected fish investigated in this research. Thirteen parasites species belong to the phylum monogeneans with *P. barbi* recorded the highest prevalence rate. Luque and Poulin, (2007) and Bellay *et al.* (2015), reported that monogeneans are the fish parasites that are dominant in the fish parasites community in all tropical reservoirs. Furthermore, *P. barbi* is a generalist parasite that infects most fish species from the family Cyprinidae, *D. lampam* is host specialist parasite on *Barbonymus* species and *C. sundanensis* is also host specialist parasite on *H. numerus* (Bu and Leong, 1997).

Similarly, the fifteen infected fish species out of 17 infected fish analysed belong to the family Cyprinidae with *B. Schwanefeldii* recording the highest number of in the reservoirs. Most of the fish parasites in the reservoirs are parasites that infect fish hosts belong to the family Cyprinidae as reported by Bu and Leong, (1997). *B. Schwanefeldii* is one of the most common and widely distributed freshwater fish in the reservoirs (Mohd *et al.*, 2012).

CONCLUSION AND RECOMMENDATION

A 32 species of external and internal parasites of the freshwater fishes were isolated in Temengor, Bersia and Chenderoh Reservoirs. Three new recorded fish parasites species (*Balantidium* sp., *Myxobulus* sp. and *Henneguya* sp.) were reported for the first time in the reservoirs. Majority of the parasite species identified in the study were dominated by monogeneans. Many parasites' species are yet to be identified, A more advance techniques/molecular techniques be used on the identification of the numerous species of parasites in the reservoirs**.** Changing of fish fauna and parasites cause by the introduction of non-native fish species with their parasites by incidental release or escape from the fish farms in the river is another concern.

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