UNIVERSITI PUTRA MALAYSIA

DETECTION OF PARASITES IN BUTTERFLY PEACOCK BASS, *Cichla ocellaris* CAPTURED FROM TASIK PRIMA, PUCHONG, SELANGOR

NURUL SYAZWANI BINTI ABDUL RAHIM CHOO

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FACULTY OF VETERINARY MEDICINE

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NURUL SYAZWANI BINTI ABDUL RAHIM CHOO

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CERTIFICATIONS

It is hereby certified that we have read this project paper entitled "Detection of Parasites in Butterfly Peacock Bass, *Cichla ocellaris,* Captured from Tasik Prima, Puchong, Selangor", by Nurul Syazwani Binti Abdul Rahim Choo and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan VPD 4999 – Projek Ilmiah Tahun Akhir.

PENGESANAN PARASIT DALAM IKAN RAJA, *Cichla ocellaris* YANG DITANGKAP DARI TASIK PRIMA, PUCHONG, SELANGOR.

Oleh

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Ikan a makanan sumber protein tinggi dan merupakan salah satu makanan yang paling mudah diperoleh, menjadikannya disukai ramai. Walau bagaimanapun, jangkitan parasit seperti protozoa, krustasea serta helmin adalah perkara biasa pada ikan, di mana ia boleh menimbulkan risiko penularan penyakit kepada manusia. Ikan Raja (*Cichla ocellaris*), ialah spesies ikan invasif dan pemangsa yang berasal dari Sungai Amazon dan popular di kalangan pemancing sebagai ikan sukan di Malaysia. Walaupun ada yang mengamalkan menangkap dan melepaskan sebagai hobi, segelintir yang lain menangkap untuk konsumi mereka sendiri. Kekurangan kajian tentang *C. ocellaris* menjadikannya satu cabaran kerana ia menimbulkan peluang untuk penyakit zoonotik untuk muncul. Oleh itu, tujuan kajian ini adalah untuk mengesan kehadiran parasit dalam *C. ocellaris* yang ditangkap dari Tasik Prima, iaitu bekas kolam lombong di Puchong, Selangor. Sebanyak 10 ekor *C. ocellaris* terdiri daripada juvana dan dewasa (n=5/kumpulan) telah ditangkap dari Tasik Prima, Puchong. Ikan tersebut telah diperiksa untuk ekto- dan endoparasit. Sampel dari kulit, insang, dan usus telah dikumpul dan diperiksa melalui teknik lekapan basah terus dan

diperhatikan di bawah mikroskop kompaun in-situ. Parasit yang dikenal pasti ialah *Dactylogyrus sp.,* dan trematod digenetik. Jumlah kelaziman parasit untuk ikan dewasa dan ikan juvana *C. ocellaris* adalah 70% (3/5 daripada ikan dewasa dan 4/5 daripada ikan juvana). Ektoparasit *Dactylogyrus sp*. hanya dijumpai di dalam 40% (2 daripada 5) *C. ocellaris* dewasa. Jumlah kelaziman "encysted metacercariae" (EMC) dalam filamen insang untuk kedua-dua ikan dewasa dan ikan juvana adalah 70% (3/5 daripada ikan dewasa dan 4/5 daripada ikan juvana). Intensiti ektoparasit adalah yang paling tinggi dalam insang untuk kedua-dua ikan juvana dan ikan dewasa dengan 2.5 dan 52.6 masing-masing. Kesimpulannya, kajian ini mendedahkan bahawa tidak terdapat beban ketara jangkitan ekto- dan/atau endoparasit antara ikan dewasa dan ikan juvana *C. ocellaris* yang ditangkap dari Tasik Prima, Puchong. Dapatan kajian ini boleh digunakan sebagai data asas untuk penyiasatan lanjut mengenai jangkitan parasit *C. ocellaris* di Malaysia.

Kata kunci: *Cichla ocellaris*; Ikan Raja; Tasik Prima, Puchong; kelaziman; ektoparasit; endoparasit

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD4999 – Final Year Project.

DETECTION OF PARASITES IN BUTTERFLY PEACOCK BASS, *Cichla ocellaris* CAPTURED FROM TASIK PRIMA, PUCHONG, SELANGOR

By

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Fish being a high-protein source food and is one of the easiest foods to obtain, making it favorable to many. However, parasitic infection such as protozoa, crustacean as well as helminths are common in fish, which can greatly pose a risk of transmitting diseases to humans. Butterfly Peacock Bass (*Cichla ocellaris*), is an invasive and a piscivorous fish species that originates from the Amazon River and is popular among anglers as sport fish in Malaysia. While some practice catch and release as a hobby, others catch for their own consumption. The lack of study on *C. ocellaris* makes it a challenge as it raises an opportunity for zoonotic diseases to emerge. Thus, the purpose of this study was to detect the presence of parasites in *C. ocellaris* sampled from Tasik Prima, an ex-mine pool in Puchong, Selangor. A total of 10 *C. ocellaris*, consisting of juveniles and adults (n=5/group) were caught from Tasik Prima, Puchong. They were examined for ecto- and endoparasites. Samples from the skin, gills, and intestines were collected and examined via direct wet

mount technique and observed under the compound microscope in-situ. The parasites identified were *Dactylogyrus sp*., and digenetic trematode. Total prevalence of parasites for both adults and juveniles *C. ocellaris* was 70% (3/5 in adults and 4/5 in juveniles). Ectoparasite *Dactylogyrus sp.* was only observed in 40% (2 of 5) of adult *C. ocellaris*. Total prevalence of encysted metacercariae (EMC) in gill filaments for both adult and juvenile was 70% (3/5 in adults and 4/5 in juveniles). Intensity of ectoparasite was the highest in gills for both juveniles and adults with 2.5 and 52.6 respectively. In conclusion, this study revealed that there is no significant burden of ecto- and/or endoparasite infection between adult and juvenile *C. ocellaris* captured from Tasik Prima, Puchong. The findings of this study can be used as a baseline data for further investigation on parasitic infestation in *C. ocellaris* in Malaysia.

Keywords: *Cichla ocellaris*; Butterfly Peacock Bass; Tasik Prima, Puchong; prevalence; ectoparasites; endoparasites

1.0 INTRODUCTION

1.1 STUDY BACKGROUND

Butterfly Peacock Bass, *(Cichla ocellaris)* or locally known as "Ikan Raja" is an invasive and a piscivorous fish that originates from the Amazon River in tropical South America (Kullander *et al.*, 2007). It is usually found in freshwater where the environment is rather stagnant and consists of shady areas. The introduction of *C. ocellaris* in Malaysia back in the early 1990's by a fish breeder from Perak (Wong, 1996) has caused a threat to native biodiversity (Reiss *et al.*, 2012).

Tasik Prima, an ex-mining land in Puchong, Selangor that now serves as an open recreational lake that is famous amongst local anglers. *C. ocellaris* is popular among anglers as a sport fish, where it is noticed that most anglers in Malaysia engage in catch and release of peacock bass as a hobby, while others were seen engaging in catch and sell or/and consume *C. ocellaris* (Khaleel *et al.*, 2021) which indirectly have contributed to its success in reproducing and invading new habitat as well as the opportunity for zoonotic diseases to emerge.

However, there is very limited understanding and research available on this species as it is a less popular freshwater fish species captured for consumption and is often overlooked. Hence, the information pertaining to the parasite burden in this species is not well known. Thus, this project can act as a preliminary study to detect parasitic infections in *C. ocellaris* captured from Tasik Prima, Puchong.

Fish is a high-protein source food and is one of the cheapest and easiest foods to obtain, making it within the reach of most average people (Mohanty, 2015). In Malaysia, the average per capita fish consumption is at 42.7 kg per year (MAFI, 2020). However, parasitic

infections are common in fish such as protozoa, crustacean as well as helminths, which can greatly pose a risk of transmitting diseases to humans. Endoparasites such as digenetic trematodes are a diverse group of parasites that rely on more than one host to complete their life cycle. They can usually be seen in larval or adult forms and mostly exist as endoparasites. Currently, Asian countries are facing public health problems relating to fish-borne zoonotic (FBZ) through trematode infections (Murrell *et al.,* 2007; Tran *et al.,* 2008; Phan *et al.,* 2010).

Ectoparasites usually harbor on the skin, fin as well as the gills of the fish. Fish ectoparasites are common, but some ectoparasites are able to cause serious problems especially in juvenile fish (Demopoulos and Sikkel, 2015). Ectoparasite infestation can cause ulcerations and abrasions that will lead to secondary infections. Generally, parasite infections can affect the health, survival and marketability of the infected fish. Indirectly, ectoparasites open a clear path for possible zoonotic diseases to emerge. Thus, the findings of this study can be used as a baseline data for further investigation on parasitic infections in *C. ocellaris* in Malaysia and the potential public health importance.

1.2 HYPOTHESIS

Null Hypothesis 1: There is no significant association between ecto- and/or endoparasite infection between adult and juvenile *C. ocellaris* captured from Tasik Prima, Puchong.

Alternative Hypothesis 1: There is significant association between ecto- and/or endoparasite infection between adult and juvenile *C. ocellaris* captured from Tasik Prima, Puchong.

Null Hypothesis 2: There is no significant finding of blood parasite in adult and juvenile *C. ocellaris* captured from Tasik Prima, Puchong.

Alternative Hypothesis 2: There is significant finding of blood parasite in adult and juvenile *C. ocellaris* captured from Tasik Prima, Puchong.

1.3 OBJECTIVES

Objective 1: To detect parasites on the gills, skin and intestine of adult and juvenile *C. ocellaris* captured from Tasik Prima, Puchong

Objective 2: To detect blood parasites of adult and juvenile in *C. ocellaris* captured from Tasik Prima, Puchong.

2.0 LITERATURE REVIEW

2.1 BUTTERFLY PEACOCK BASS (*Cichla ocellaris*)

Peacock Bass is listed under Invasive Alien Species in 2018 (NCIASM, 2018). Invasive Alien Species (IAS) according to Convention on Biological Diversity (CBD) is "*A species, subspecies or lower taxon introduced outside its natural past or present distribution, includes any part, gametes, seed, eggs or propagules of such species that might survive and subsequently reproduce*" (CBD, 2002). For years, the involvement of humans in the migration of fish across various places has led to a significant alteration in terms of introduction of alien species and impacting the indigenous species (Callaway and Maron, 2006; Yong *et al.*, 2014; Fazhan *et al.*, 2017). It was believed that the introduction of Peacock

Bass in Malaysia started in the early 90's by a fish breeder in Perak (Wong, 1996).

Recent study showed that Peacock bass has successfully invaded in the entirety of Peninsular Malaysia, with currently no documented sighting of its presence in Kelantan, Sabah, and Sarawak (Khaleel *et al.*, 2021). Among the common Peacock Bass species that was reported in Malaysia was Butterfly Peacock Bass (*Cichla ocellaris*) (Chong *et al.,* 2010). Even with the scarce understanding and research pertaining to parasite burden in Peacock Bass, let alone in this species (*C. ocellaris)*, according to Perlis State Fisheries Department, locals were encouraged to catch and consume Peacock Bass as an effort to reduce its population (New Straits Times, 2017). Peacock Bass has gained its popularity in Malaysia among local anglers because of its aggressive nature in striking baits indirectly impacting the biodiversity in Malaysia, particularly in freshwater ecosystems (Yamada and Takemoto, 2013).

2.2 PARASITES OF FRESHWATER FISH

Parasitic infection is more common in the wild than in hatcheries and captivity. High intensity of parasites in a fish can alter the physical, physiological and reproductive systems of fishes (Iwanowicz, 2011). Parasitic infections are common in fishes and they serve as potential hosts for zoonotic diseases. Wild fish species have a high chance of parasitic infestation, and mostly, the host does not show any noticeable effect (Oghenochuko *et al*., 2020). Endoparasites are parasites that harbor the muscles and internal cavities of an organism (Roberts and Janovy, 2005). Unlike ectoparasites, endoparasites have the capability to infect humans when they consume raw or improperly prepared fish and, thus, raising public health concerns (Hoffman, 1998). Common examples of endoparasitic groups are helminths such as trematodes, cestodes and nematodes.

A study by Macmillan (1991) revealed that ectoparasites are the most common parasites infesting fishes reared in ponds and aquariums. Ectoparasites are parasites that harbor on the external surfaces of the fish (Roberts and Janovy, 2005). The areas included are the skin, fins, gills as well as the oral cavity. These parasites will usually cause skin irritation and increase secretion of mucus (Mehdizadeh-Mood *et al.*, 2011) which can then lead to secondary infection by bacteria or fungus and reduce growth, reproduction and cause high morbidity in fish (Pike, 1989). Some examples of ectoparasite in freshwater fish include *Dactylogyrus sp, Gyrodactylus sp, Trichodina sp,* and *Argulus sp.* (Klinger *et al*., 2013)

2.3 PUBLIC HEALTH CONCERNS

Fish is a cheap and easy high-protein source of food, but fish are also known to be prone to parasitic infestation especially in wild populations where it is less studied. Diseases linked with trematodes are often zoonotic such as *Opisthorchis spp.* (liver fluke) and *Heterophyes* (intestinal fluke) (Madsen *et al.*, 2022). This is due to the consumption of contaminated fish which can possibly lead to serious diseases (Sichewo *et al*., 2013). Fish can get infested with either ecto- or endoparasite and it has been reported that endoparasites can cause more damage to their hosts than ectoparasites (Ashraf and Zafar 2013). For instance, studies by Chen *et al*. (1991) and De & Le (2001) have reported some cases of fish-borne zoonotic (FBZ) by the metacercariae of *Centrocestus sp.* that can usually be found encysted in the gills of the fish. In cases of humans infected with this parasite, mild symptoms such as epigastric discomfort, indigestion as well as diarrhea can be seen (Hong *et al*., 1989).

3.0 MATERIALS AND METHODS

3.1 FISH SAMPLING

A total of 10 Butterfly Peacock Bass (*Cichla ocellaris*) consisting of 5 juveniles (< 20cm) and 5 adults (> 20cm) were collected for this study. Specimens were captured from an ex-mining lake, Tasik Prima, Puchong by casting technique using fishing rods with live feeder baits such as juvenile tilapia bought from an aquarium shop. The fish was kept alive and transported to a dissecting station that was set up on land by boat in a temporary aerated transport tank until it was dissected under IACUC approval (UPM/IACUC/AUP-U004/2023). Water quality test was also done using API® freshwater master test kit following the manufacturer's guidelines.

3.2 SPECIMEN EXAMINATION

The fish was restrained using a damp cloth to prevent drying of the skin and was then immobilized via pithing method. The weight and total body length was measured and recorded. External examination of the fish was done to examine for any visible ectoparasites; in this case, no visible ectoparasite was observed. Firstly, blood sample was collected via the caudal vein just below the lateral line at the peduncle region using a 1ml syringe and a 25G/23G needle for juvenile and adult, respectively. The blood samples were processed using the thin blood smear method and observed under compound microscope in-situ.

Then, scraping samples were obtained from the skin starting from behind the pectoral fins towards the caudal of the fish. The sample was then placed on a glass slide and stained with methylene blue for parasite identification by microscopic observation in-situ. Next, the

operculum was cut open to expose the gills and visual examination was done to assess for any visible ectoparasites before dissecting approximately 0.5cm^2 of one gill arch and the sample was placed on a glass slide. The gill filaments were then spread out to minimize overlapping for better visualization before the sample was stained with a drop of methylene blue and covered with a cover slip for microscopic examination.

Following that, necropsy was done where the fish was dissected starting from the anterior to the vent opening and moved cranially along the medium ventral line towards the operculum using scissors. Then, a vertical cut was done towards dorsally reaching approximately the level of the lateral line and continued with an oblique caudal direction following the dorsal edge of the body cavity to reach the starting cut which was the anterior of the vent opening. The flap was then removed to expose the abdominal cavity and organs without manipulation for assessments. The intestines were then removed and extended on a dissecting tray. Using a scissor, the intestines were cut open longitudinally and scanned for any macroscopic parasites. Scraping samples of the intestinal walls were obtained, placed on a glass slide, stained with a drop of methylene blue, covered with a cover slip and observed under compound microscope in-situ.

3.3 PARASITE IDENTIFICATION, PREVALENCE AND INTENSITY

Parasite identification was done using a compound microscope in-situ based on the pictorial guide by Klinger *et al*., (2013), Pouder *et al*., (2020) and Floyd *et al.,* (2023). Once parasites were identified, pictures were captured and data were tabulated. The prevalence and intensity of the parasites observed were calculated using a formula adapted from Mamani *et al* (2004).

$$\text{Prevalence (\%)} = \frac{\sum \text{fish infected by parasites}}{\sum \text{fish examined}} \times 100\%$$

$$\text{Intensity} = \frac{\sum \text{parasite found}}{\sum \text{infected fish}}$$

3.4 DATA ANALYSIS

All data were tabulated using Microsoft Excel and statistical analysis was done by using IBM SPSS Statistical Analysis Version 27. Chi-Squared was used to test the prevalence of parasites between adult and juvenile fish. Shapiro-Wilk test was used to test the normality of the intensity of parasites between sampled organs which were the gills, skin and intestines in juvenile and adult fish. The data obtained did not pass the normality test and thus, Mann-Whitney U test was selected as the non-parametric analysis.

4.0 RESULTS

4.1 WATER QUALITY TEST

All of the water quality parameters results were normal and are presented in Table 1.0.

Table 1.0 Water quality parameters from different sampling spots at Tasik Prima, Puchong.

Parameters	Sampling spot 1	Sampling spot 2	Sampling spot 3
Temperature (°C)	30.8	30.7	30.2
Dissolved oxygen (mg/l)	5	6	4
pH	8.0	8.0	8.0
Ammonia (ppm)	0	0	0
Nitrite (ppm)	0	0	0
Nitrate (ppm)	0	0	0
Salinity (ppt)	0.11	0.11	0.11

4.2 PREVALENCE OF ECTOPARASITES

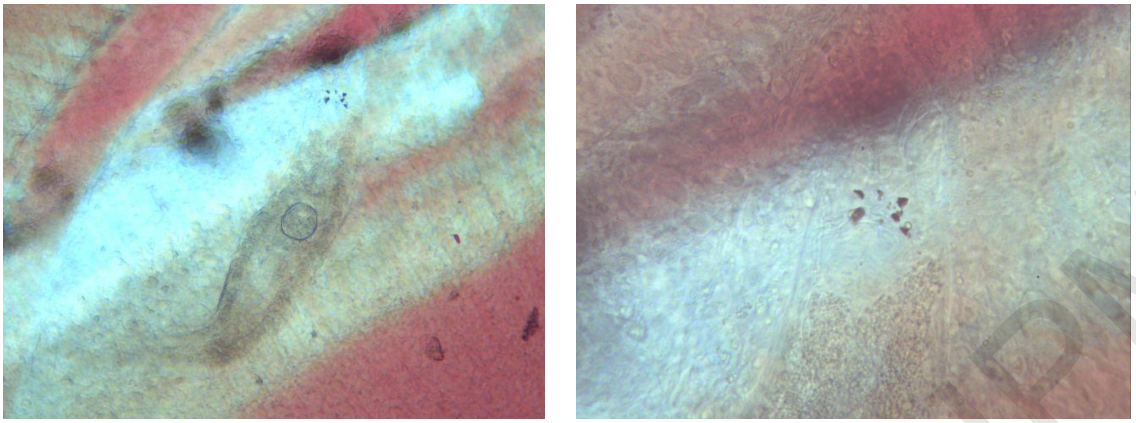

Figure 1.0: The figures showed *Dactylogrus sp*. found in the gills of adult *C. ocellaris* captured from Tasik Prima, Puchong.

Results of prevalence of ectoparasite in this study are presented in Table 4.0. Ectoparasite identified was *Dactylogyrus sp*. that was only found in the gills of adult *C. ocellaris* and none in juveniles. Based on the results obtained, out of 5 adult samples, 2 fish were infected and the prevalence of *Dactylogrus sp*. infected fish was 40%.

Table 2.0 Prevalence of ectoparasites (*Dactylogyrus sp.*) in Adult *C. ocellaris*

Ectoparasite	**No. of infected fish**	**No. of examined fish**	**Prevalence (%)**
Dactylogyrus sp.	2	5	40.0

4.3 PREVALENCE OF ENDOPARASITES

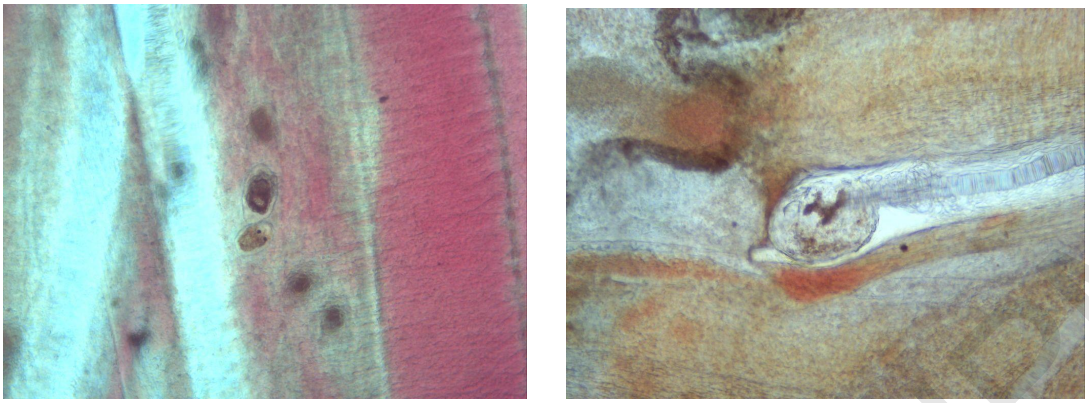

Figure 2.0: The figures showed encysted metacercariae (EMC) of digenetic trematode in the gills of both adult and juvenile *C. ocellaris* captured from Tasik Prima, Puchong. EMC observed in adult *C. ocellaris* (left). EMC observed in juvenile *C. ocellaris* (right).

The overall prevalence of infection with encysted metacercariae was 70%. Figure 2.0 shows two different genus of encysted metacercariae (EMC) that were found in the gills of adult and juvenile *C. ocellaris,* respectively. Based on the results obtained, the EMC observed in adult *C. ocellaris* were unidentified as more in-depth study needs to be done to further determine the genus and species. Out of 5 samples, 3 fish were infected (60%). While the EMC found in juveniles were identified from the genus *Centrocestus spp.*, and out of 5 samples, 4 of the fish were infected (80%).

Table 3.0 Prevalence of endoparasites in Adult *C. ocellaris*

Endoparasite	**No. of infected fish**	**No. of examined fish**	**Prevalence (%)**
Encysted metacercariae (EMC)	3	5	60

Table 4.0 Prevalence of endoparasites in Juvenile *C. ocellaris*

Endoparasite	No. of infected fish	No. of examined fish	Prevalence (%)
Encysted metacercariae (EMC); *(Centrocestus sp.)*	4	5	80

4.4 INTENSITY OF PARASITES BETWEEN SAMPLED ORGANS

The results of intensity of parasites between sampled organs in this study are presented in Table 5.0 and Table 6.0. Based on the results obtained, for both adult and juvenile *C. ocellaris*, gills yielded the highest parasite intensity than the skin and intestines with 52.67 and 2.5 individuals per tail, respectively.

Table 5.0 Intensity of parasites between sampled organs in Adult *C. ocellaris*

Organs	Parasites		Total parasite	Total infected fish	Intensity (ind/tail)
	Dactylogyrus sp.	***EMC***			
Skin	-	-	-	-	-
Gill	4	154	158	3	52.67
Intestine	-	-	-	-	-

Table 6.0 Intensity of parasites between sampled organs in Juvenile *C. ocellaris.*

Organs	Parasites		Total parasite	Total infected fish	Intensity (ind/tail)
	Dactylogyrus sp.	***EMC***			
Skin	-	-	-	-	-
Gill	-	10	10	4	2.5
Intestine	-	-	-	-	-

5.0 DISCUSSION

The result from this study revealed that Butterfly Peacock Bass (*Cichla ocellaris*) captured from Tasik Prima, Puchong was infected with ectoparasite *Dactylogyrus sp.*, with the prevalence of 40% in adult *C. ocellaris* and none in juveniles. The reasons can be influenced by several variables such as host age, host sex, season as well as the presence or absence of other parasite species. This can be supported by a study by Jarkovsky (2004), in which monogenean species such as *Dactylogyrus sp*. Was found to be host specific and in another study by Kennedy (1975) who discovered that the bigger the size or age of the fish, it increases the contact time between the fish and the parasites, hence, it was only found in adult fish. This is also in agreement with a study by Untergasser (1989) that stated a bigger fish provides more space for parasite attachment, thus infecting the adults.

Endoparasite infections have always been a concern as it may potentially be zoonotic. Based on the results obtained, the prevalence of endoparasite was higher in juveniles than in adults *C. ocellaris* with 80% and 60%, respectively. This can be supported from a study by Saad *et al*. (2019) where he stated that younger fish are much more susceptible to endoparasite infections than a larger fish. This may be caused by the poorly developed immune system in younger fish. From this study, there were two different genus of metacercariae that were present in *C. ocellaris* captured from Tasik Prima, Puchong. The endoparasite that was found in juvenile *C. ocellari*s was identified from the genus *Centrocestus sp*. It is a digenetic trematode with a complex life cycle, involving invertebrate and vertebrate as hosts, in which humans are included. The metacercariae are infective to humans upon ingestion of raw or undercooked fish in dishes such as koi-pla, a dish from Thailand. There have been few reported cases in China and Vietnam of humans that consume raw fish meat and get infected with *Centrocestus sp.* (Chen et al., 1991; De & Le, 2021).

On the other hand, the overall parasite intensity between sampled organs was highest

in the gills for both adult and juvenile *C. ocellaris* with 52.67 and 2.5 individuals per tail, respectively. This could be because the gills are easy to infest as it is exposed to the external environment given its function as the center of filter feeding and are the sites of gaseous exchange. This assumption is supported by Rohde (1984) where it was stated that parasites have a tendency to reside in organs where they offer ease of occupation, sufficient space and nutrients for parasite growth and reproduction. In a study by Kearn (2004), it was discovered that fish gills serve as the most preferred habitats of many external parasites such as monogeneans.

Blood screening is a highly effective bio-indicator for fish health; a few examples of freshwater fish blood parasites include *Trypanosoma* and *Babesiosoma* (Alhayali *et al.*, 2023). Based on the results obtained, there were no significant findings of blood parasite in *C. ocellaris* captured from Tasik Prima, Puchong.

6.0 CONCLUSION

To conclude, Butterfly Peacock Bass (*C. ocellaris*) captured from Tasik Prima, Puchong were infested by ectoparasites such as *Dactylogyrus sp.* From all 10 samples obtained, *Dactylogyrus sp.* was observed only in adult *C. ocellaris* with 40% parasite prevalence. While, endoparasites such as digenetic trematodes were observed in both adult and juvenile fish with the prevalence of 60% and 80%, respectively. The parasite intensity between sampled organs for both adult and juvenile fish were both the highest in gills with 52.6 and 2.5, respectively. Also, there were no findings on blood parasites in this study. Results from this study shows that there is no significant association between the presence of parasites in adult or juvenile *C. ocellaris* captured from Tasik Prima, Puchong.

7.0 RECOMMENDATIONS

For further study to be conducted, a larger fish sample size is recommended so that a higher accuracy of results for the study of parasites in *C. ocellaris* can be obtained. This is due to the fact that a smaller sample size can have a high influence on the parasite prevalence and intensity. Also, extending the study period can help tremendously in determining what genus and species of parasites that were found as well as provide a much more organized study method and process. Not only that, by extending the study period, it allows us to explore and conduct this study in other locations for us to compare and record. Other than that, it would be more beneficial to include more organs so that a much meaningful parasitic study in this fish species can be conducted and not only focusing on certain organs. Given there is a lack of study of parasitic infestations done particularly in this fish species in Malaysia even with its rising population, thus, a much more thorough study needs to be done to have a better understanding of the possible public health impact it may pose to humans that consume this species.

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9.0 APPENDICES

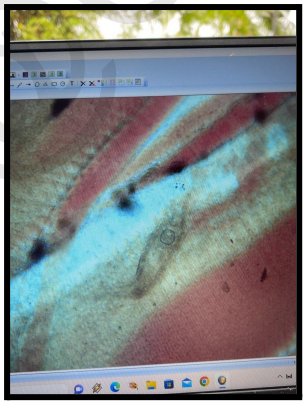
9.1 Appendix 1

Fish sampling - Captured *Cichla ocellaris* placed in a temporary aerated tank.

9.2 Appendix 2

Specimen examination - Gross observation, weight and measure body size and length, taking samples from skin, gills and intestines.

9.3 Appendix 3



Parasite observation and identification in-situ