



UNIVERSITI PUTRA MALAYSIA

**OCCURRENCE OF ECTOPARASITES IN SEA CATFISH,
HEXANEMATICHTHYS SAGOR (HAMILTON, 1822) IN SABAK
BERNAM, SELANGOR**

NUR FARHAH SOFIA BINTI MOHD NOOR SAM

**Ip
FPV 2022 7**

OCCURRENCE OF ECTOPARASITES IN SEA CATFISH, *Hexanematichthys sagor* (HAMILTON, 1822) IN SABAK BERNAM, SELANGOR

NUR FARHAH SOFIA BINTI MOHD NOOR SAM

**FACULTY OF VETERINARY MEDICINE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR**

OCCURRENCE OF ECTOPARASITES IN SEA CATFISH, *Hexanematichthys*

***sagor* (HAMILTON, 1822) IN SABAK BERNAM, SELANGOR**



NUR FARHAH SOFIA BINTI MOHD NOOR SAM

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE

FACULTY OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan.

December 2022

CERTIFICATIONS

It is hereby certified that we have read this project paper entitled “Occurrence of ectoparasites in sea catfish, *Hexanematchthys sagor* (Hamilton, 1822) in Sabak Bernam, Selangor” by Nur Farhah Sofia Binti Mohd Noor Sam and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.



DR NUR DIYANA MOHAMAD TAHIR
DVM (UPM), PhD (UPM)

Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

DR NOR AZLINA ABDUL AZIZ
DVM (UPM), PhD (BRISTOL)

Senior lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-supervisor)

DR MOHD FUAD BIN MATORI
DVM (UPM)

Veterinary Officer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-supervisor)

DEDICATIONS

To the 16-year-old Sofie that was full of dreams and hope; we made it this far. Do not give up yet okay.



ACKNOWLEDGEMENTS

First and foremost, I would like to thank Allah S.W.T for giving me the patience and strength to complete my final year project.

These five weeks of FYP definitely was not an all-smooth sail; therefore, I would like to express my gratitude to my wonderful supervisor, Dr Diyana and my co-supervisors; Dr Azlina and Dr Fuad for all of their amazing support and guidance throughout this project. I am honored to be supervised by them and I wish the best success in their life.

Of course, my FYP mate, the one I endured this project with, Intan Hazimah. I cherished every day we spent together completing our project, each day felt like hanging out with my bestie and would not have done this without her.

To my close friends;

The ones who I have been knowing since asasi; Azren, Azira, Tasha, Maria & Ikha, thank you for all these years together, I am so proud of us. To Rumah Surga homies, thank you for allowing me to be a part of you guys and for all the endless chaotic moments. To Rose, Syazreen and Mira, thank you for being the safe space I needed. To friends who constantly supported me from far, Aqilah Ujang, Izzati Hazizi and Zairul Umairah, thank you for always reassuring me and being my support system.

I would also like to thank all the staffs who helped me endlessly especially En Azmi from AAHU and Puan Latiffah from histopathology lab; your bright personality and kindness definitely helped me throughout the project. To my friends who spent days in AAHU together, thank you for making the days there more bearable and all the best for our future endeavors. To DVM2023 who helped and supported me throughout this journey, thank you and I cannot wait to see how we turn out in the next years. To my seniors and juniors who cheered me all this time, may your life be surrounded with amazing people who support you always.

Last but not least, to my family and cats (Pororo, Khumba & Lee) thank you for the support and for being there to hear my endless complaints and rants, I hope I can continue to become the Sofia you are happy and proud with.

TABLE OF CONTENT

TITLE	II
CERTIFICATIONS.....	III
DEDICATIONS	V
ACKNOWLEDGEMENTS.....	VI
TABLE OF CONTENT.....	VII
LIST OF FIGURES	IX
LIST OF TABLES	X
ABSTRAK	XI
ABSTRACT	XIII
1.0 INTRODUCTION.....	1
1.1 BACKGROUND	1
1.2 HYPOTHESIS	2
1.3 OBJECTIVES	2
2.0 LITERATURE REVIEW.....	3
2.1 SAGOR SEA CATFISH.....	3
2.2 SABAK BERNAM.....	3
2.3 ECTOPARASITES OF MARINE FISHES	3
2.4 PUBLIC HEALTH CONCERNS	4
3.0 MATERIALS AND METHODS.....	5
3.1 FISH SAMPLE	5
3.2 EXAMINATION OF THE SPECIMENS	5
3.3 IDENTIFICATION, PREVALENCE AND INTENSITY OF ECTOPARASITES ...	6
3.4 HISTOPATHOLOGICAL ANALYSIS	6

3.5 DATA ANALYSIS.....	7
4.0 RESULTS	9
4.1 WATER QUALITY TEST.....	9
4.2 PREVALENCE AND INTENSITY OF ECTOPARASITES.....	9
4.2.1 PREVALENCE AND INTENSITY OF ECTOPARASITES BASED ON SAMPLE SITE	11
4.3 HISTOPATHOLOGY ANALYSIS.....	13
4.3.1 HISTOPATHOLOGY RESULT OF JUVENILE FISH.....	14
4.3.2 HISTOPATHOLOGY RESULTS OF ADULT FISH.....	14
5.0 DISCUSSION	19
6.0 CONCLUSION.....	22
7.0 RECOMMENDATIONS.....	22
8.0 REFERENCES.....	24

LIST OF FIGURES

Figure 1.0	Criteria of histopathology scoring of gills	7
Figure 2.0	<i>Trichodina sp.</i> on skin and gills of <i>Hexanematichthys sagor</i>	10
Figure 2.1	<i>Argulus sp.</i> in gills of <i>Hexanematichthys sagor</i>	10
Figure 2.2	<i>Dactylogyrus sp.</i> in gills of <i>Hexanematichthys sagor</i>	10
Figure 2.3	<i>Caligus sp.</i> in skin of <i>Hexanematichthys sagor</i>	11
Figure 3.0	Intensity of ectoparasites according to respective sites in juvenile and adult <i>Hexanematichthys sagor</i>	13
Figure 4.0	Histopathological scoring of gills for juvenile and adult <i>Hexanematichthys sagor</i>	15
Figure 4.1	Percentage of lamellar hyperplasia of gills	16
Figure 4.2	Percentage of ancillary criterion of gills	16
Figure 5.0	Parasites observed in histopathology evaluation of gills	17
Figure 5.1	Parasitic cyst observed in histopathology evaluation of gills	17
Figure 5.2	Cellular anomalies in histopathology of gills	18

LIST OF TABLES

Table 1.0	Water quality parameters of water sample from sampling location	9
Table 2.0	Prevalence and intensity of ectoparasites in juvenile <i>Hexanematichthys sagor</i>	11
Table 2.1	Prevalence and intensity of ectoparasites in adult <i>Hexanematichthys sagor</i>	11
Table 3.0	Prevalence and intensity of ectoparasites in juvenile <i>Hexanematichthys sagor</i> based on sample site	12
Table 3.1	Prevalence and intensity of ectoparasites in adult <i>Hexanematichthys sagor</i> based on sample site	12
Table 4.0	Histopathology scoring of gills in juvenile <i>Hexanematichthys sagor</i>	14
Table 4.1	Histopathology scoring of gills in adult <i>Hexanematichthys sagor</i>	14

ABSTRAK

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

KEJADIAN EKTOPARASIT DALAM IKAN KELI LAUT, *Hexanematichthys sagor* (Hamilton, 1822) DI SABAK BERNAM, SELANGOR.

Oleh

Nur Farhah Sofia Binti Mohd Noor Sam

No. Matrik: 199130

2022

Penyelia: Dr Nur Diyana Binti Mohamad Tahir

Penyelia bersama: Dr Nor Azlina Binti Abdul Aziz & Dr Mohd Fuad Bin Matori

Ikan keli laut, *Hexanematichthys sagor* (Hamilton, 1822) biasa ditemui di muara sungai di Malaysia dan kadangkala ditangkap untuk dimakan oleh penduduk tempatan. Perilaku makan ikan ini menimbulkan kebimbangan kerana ia berpotensi untuk dijangkiti parasit dan boleh membawa kepada isu kesihatan awam. Oleh itu, tujuan utama kajian ini adalah untuk menilai kejadian ektoparasit, membandingkan kejadian ektoparasit antara ikan juvenil dan dewasa serta menilai perubahan histopatologi pada kulit, insang dan sirip. Sebanyak lima ekor ikan juvenil dan lima ikan dewasa berjaya diporeleh daripada nelayan tempatan di Sekendi, Sabak Bernam. Ektoparasit daripada kulit, insang dan sirip dikenal pasti menggunakan teknik lekapan basah dan insang diperolehi untuk penilaian histopatologi dan diawetkan dalam 10% formalin penimbal yang disejukkan untuk diproses. Kelaziman dan keamatan parasit dianalisis menggunakan ujian-t dalam GraphPad versi 9. Ektoparasit yang dikenal pasti oleh ialah

Trichodina sp., *Dactylogyruis sp.*, *Argulus sp.* dan *Caligus sp.* Dalam kesemua 10 sampel ikan, *Trichodina sp.* telah diperhatikan dengan prevalens 100% dan *Dactylogyruis sp.*, diperhatikan dalam semua ikan dewasa. Keamatan parasit adalah yang tertinggi dalam insang untuk kedua-dua juvenil dan dewasa; 11.0 dan 23.2 masing-masing. Bagi perubahan histopatologi insang, pemarkahan 0-3 diukur berdasarkan lima kriteria iaitu hiperplasia lamellar, lakuran lamellar, anomali sel, edema lamellar dan kriteria sampingan, iaitu ketiadaan atau kehadiran parasit. Daripada kajian ini, hiperplasia lamellar diperhatikan dalam 90% sampel insang dan 80% mempunyai kriteria sampingan. Walau bagaimanapun, kajian ini mendedahkan bahawa tiada perbezaan yang signifikan dalam beban parasit antara ikan juvenil dan dewasa. Penemuan yang diperolehi daripada kajian ini boleh berfungsi sebagai inventori parasit dan untuk kajian masa depan.

Kata kunci: *Hexanematchthys sagor*; Ikan Keli Sagor; Sabak Bernam; kelaziman; ektoparasit

ABSTRACT

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

OCCURRENCE OF ECTOPARASITES IN SEA CATFISH, *Hexanemataichthys sagor* (Hamilton, 1822) IN SABAK BERNAM, SELANGOR.

By

Nur Farhah Sofia Binti Mohd Noor Sam**Matrics number: 199130****2022****Supervisor:** Dr Nur Diyana Binti Mohamad Tahir**Co-supervisors:** Dr Nor Azlina Binti Abdul Aziz & Dr Mohd Fuad Bin Matori

The sea catfish, *Hexanemataichthys sagor* (Hamilton, 1822) are commonly found in estuaries in Malaysia and occasionally caught for consumption by the locals. The scavenging feeding behavior raises concern as it has the potential to harbor parasites and can lead to public health issues. Therefore, the main purpose of this study was to evaluate the occurrence of ectoparasites, compare the ectoparasite occurrence between juvenile and adult fish and assess the histopathological changes on the skin, gills and fin. A total of five juvenile and five adult fish were collected from local fishermen in Sekendi, Sabak Bernam. Ectoparasite from the skin, gills and fin were identified using direct wet mount technique and the gills were obtained for histopathological evaluation and preserved in chilled 10% buffered formalin for processing. The parasitic prevalence and intensity were analyzed using t-test in GraphPad version 9. The ectoparasite identified by wet mount were *Trichodina sp.*, *Dactylogyrus sp.*, *Argulus sp.* and *Caligus sp.* In all 10 fish samples, *Trichodina sp.* was observed with the prevalence of 100%

and *Dactylogyrus sp.*, was observed in all adult fish. Parasite intensity was the highest in gills for both juvenile and adult; 11.0 and 23.2 respectively. For the gill histopathology changes, a scoring of 0-3 was measured based on five criteria which were lamellar hyperplasia, lamellar fusion, cellular anomalies, lamellar oedema and an ancillary criterion, which was the absence or presence of parasite. From this study, lamellar hyperplasia was observed in 90% of the gill samples and 80% had the ancillary criterion. However, this study revealed that there was no significant difference in parasitic burden between juvenile and adult fish. The findings obtained from this study of the *Hexanematichthys sagor* could serve as a preliminary parasite inventory and for future studies on the potential risks of consumption of the species.

Keywords: *Hexanematichthys sagor*; Sagor catfish; Sabak Bernam; prevalence; ectoparasites

1.0 INTRODUCTION

1.1 BACKGROUND

Hexanematchthys sagor (Hamilton, 1822) or locally known as “Duri Pedukang” (Froese & Pauly, 2022) lives in coastal environments such as estuaries and mangroves. This sea catfish is a non-commercial species occasionally caught for human consumption and sold fresh or dried. Sabak Bernam in Selangor, Malaysia is known as a coastal town with fisheries as one of its main economic activities (Baharudin et al ., 2021). Sabak Bernam is one of the areas for shrimp pond production in Selangor after Kuala Selangor and Kuala Langat districts (Mohd Fozi et al ., 2015). With increasing aquaculture activities, the impact of collateral diseases such as ectoparasite infection in wild species is still poorly understood (Bouwmeester et al ., 2021). Therefore, the sea catfish such as *Hexanematchthys sagor* could be the sentinel model in filling the knowledge voids regarding the collateral disease impacts from aquaculture and the effects of consumption of the fish.

However, there is limited information and research available on this fish species as it is a less popular species for consumption among the catfish species. Hence, the information regarding ectoparasite burden in this species is not well known. Thus, this study is to determine the occurrence of ectoparasitic infections in the *Hexanematchthys sagor*, the changes in histopathology and its potential public health importance. Ectoparasites infect parts of the fish such as on the skin, fin and gills which can cause secondary infections and lead to viral and bacterial diseases. In cultured fish, it is said that ectoparasites can impact the survivability of the fish and inhibit their growth. Although this species of sea catfish is sold non-commercially, ectoparasite infections can reduce the fish quality and lead to economic loss (Indahsari, 2019). It can be expected that the wild population of this sea catfish will carry multiple ectoparasites and histopathological changes can be observed in the tissues sampled with more parasite burden in the adults compared to the juveniles. The findings in the sea

catfish in the sampling sites in Sabak Bernam could potentially help in better understanding the effects of parasite infection and its impacts in aquaculture. The findings can serve as a preliminary parasite inventory which can be further expanded as a disease monitoring tool in surrounding farms and increase our knowledge on the impacts of aquaculture.

1.2 HYPOTHESIS

Null Hypothesis 1: There is no significant difference in parasitic burden between Juvenile & Adult *Hexanematichthys sagor*.

Alternative Hypothesis 1: There is significant difference in parasitic burden between Juvenile & Adult *Hexanematichthys sagor*.

Null Hypothesis 2: Ectoparasite infection will not cause significant histopathological changes in skin, fin & gills of the fish.

Alternative Hypothesis 2: Ectoparasite infection will cause significant histopathological changes in skin, fin & gills of the fish.

1.3 OBJECTIVES

Objective 1: To evaluate and compare the occurrence of ectoparasites on the skin, fin & gills in juvenile & adult *Hexanematichthys sagor*.

Objective 2: To evaluate the histopathological changes on the skin, fin & gills of the juvenile & adult *Hexanematichthys sagor*.

2.0 LITERATURE REVIEW

2.1 SAGOR SEA CATFISH

Hexenematchthys sagor (Hamilton, 1822), also locally known as Duri pedukang (Froese & Pauly, 2022) is a part of the Ariidae catfish family. Species from the Ariidae family mainly lives in marine water however there are also species found in freshwater and brackish water (Chong *et al.* , 2010). Similarly to other catfish species which are bottom dwellers (Afolabi *et al.* , 2020) and have scavenging feeding behavior which indicates that they feed on dead animals and it has been said that fisheries discards are important source of feed for the marine scavengers (Uhlmann *et al.* , 2019). Among the catfish species that are found in brackish water or the mangrove ecosystem, this species also has a low economic value in Malaysia (Khoo H., 1994) . This species is also known to live around the housing areas that are near estuaries and mangrove environment. Therefore, although the sagor sea catfish is not the top priority of fish catch among the locals, due to its availability, the locals might still consume it. However, there are very few studies about this species of sea catfish generally (Nasution & Machrizal, 2021), let alone about the occurrence of ectoparasites.

2.2 SABAK BERNAM

Sabak Bernam is a district that is located at the northwestern part of Selangor. Apart from the city area, this district comprises of agriculture areas as well as mangrove and coastal forests (Mohd Fozi *et al.* , 2015). In this district, agriculture activities are one of the most important economical sources as well as fishing (Baharudin *et al.* , 2021).

2.3 ECTOPARASITES OF MARINE FISHES

Fishes are also susceptible to parasitic infections similarly as other animals. Likewise, there are also endoparasites and ectoparasites. Ectoparasites are parasites that live on the external surfaces of fish which are the skin, fin and gills (Gilda D. *et al.* , 2001). The

ectoparasites that can infect fish are from the protozoan group, monogenean group and the crustacean group whether in freshwater or marine environment. Based on Klimpel S. *et al* (2019), parasites in the protozoan group are single-celled and free-living while monogeneans are oviparous and have a direct life cycle which they do not need an intermediate host. Crustaceans are one of the most varied parasitic group in the marine environment and can be categorized into further groups such as Copepoda, Branchiura and Isopoda. Generally, low ectoparasites infection would not cause a severe health issues or disruption in normal physiological function compared to other pathogen such as virus, bacteria or fungi. However, it can be the cause or entry point for those pathogens to infect the fish and lead to secondary infection (Indahsari *et al* ., 2019). Studies conducted on the occurrence of the ectoparasites has been done in several commonly cultured fish species in catfish but none has been done in the wild population.

2.4 PUBLIC HEALTH CONCERNS

If compared to endoparasites, there are less concern regarding public health issue regarding ectoparasites. However, there are some species that can infect marine fish and lead to issue for human consumption. For example, the metacercaria of *Centrocestus sp.* can be encysted in the gills of its host and not only cause gill lesions and mortality to the fish but can also cause gastrointestinal health issues in human (Pace *et al* ., 2020). Since the subject of the present study; *Hexanematichthys sagor*, habitats estuaries that are can also be main areas for fisheries as well as consist of human housing areas, the occurrence of any zoonotic potential ectoparasites can be investigate and identified for the importance of public health.

3.0 MATERIALS AND METHODS

3.1 FISH SAMPLE

A total of 10 sea catfish (*Hexenemataichthys sagor*) that consist of 5 juvenile and 5 adult specimens were collected for this study. The specimens were obtained from several sites in Sabak Bernam, Selangor by using methods such as collection from local fishermen catching with net and fishing pole. The fish was kept alive in an aerated container until it was dissected under IACUC approval (UPM/IACUC/AUP-U005/2022). Water quality test was done with API® freshwater master test kit by following the manufacturers protocol.

3.2 EXAMINATION OF THE SPECIMENS

The fish was restrained using a damp cloth to prevent the skin from drying out as well as to minimize any injuries. The dorsal and pectoral fins were trimmed of the fish by using scissors to prevent injuries to handlers. The fish was then immobilized by using decapitation and pithing method. The standard body length and total body length was measured and recorded. Then external examination of the specimen was done to examine for any lesions as well as visible ectoparasites; parasites found were placed in chilled 10% buffered formalin. If there are any lesions on the skin, the skin sample was cut using punch biopsy and placed in chilled 10% buffered formalin for histopathological examination. However, during our sampling no visible ectoparasites and skin lesions were observed, thus no skin and fin samples were taken for histopathological evaluation.

Skin and tail scraping was done where for each fish, the skin along the belly and caudal tail fin region were chosen to use as scraping sample. The sample was place on the glass slide and stained with methylene blue for parasite identification by microscopic observation.

Then, the operculum was cut open to expose the gills. Visual examination of the gills was done first followed by dissecting the filament of one gill arch from both sides of the gills approximately by 0.5cm² in-situ and placed on a glass slide. The specimen is then stained with

methylene blue for microscopic examination. Then the whole gills of one side were dissected out to be measured as well as further visual examination. A part of the gills was dissected and placed into chilled 10% buffered formalin for histopathological examination.

3.3 IDENTIFICATION, PREVALENCE AND INTENSITY OF ECTOPARASITES

Microscopic identification of the parasite was done using the light microscope based on the pictorial guide by Klimpel *et al.* (2019). After identifying the parasites, the data will be tabulated and the prevalence and intensity of the parasites will be calculated with the formula adapted from Muchlisin *et al.* (2014).

The prevalence of parasites:

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

The intensity of parasites:

$$\text{Intensity} = \sum \text{parasites found} / \sum \text{infected fish}$$

3.4 HISTOPATHOLOGICAL ANALYSIS

The gill samples were fixed in 10% buffered formalin before undergoing the standard histology process. Since there were no skin lesions observed on the fish samples, no skin specimen was taken for histopathology examination.

After the samples were fixed in 10% formalin overnight, it was then trimmed and placed into cassette and placed in a mixture solution of 10% formic acid and 10% buffered formalin for 5 hours. This step was done to decalcify the gills for histology sectioning. After the 5 hours, the gills were then washed with running tap water for 30 minutes before washing with 70% alcohol for 5 minutes. After the 5 minutes, the samples were then placed in 70% alcohol and sent to the histopathological laboratory to be processed. Histology process can be referred to appendix.

Histopathology scoring of the gills were done based on five criteria which are lamellar hyperplasia, lamellar oedema, lamellar fusion, cellular anomalies and an ancillary criterion.

This method of scoring was adapted from Mitchell *et al* . (2012) as stated below in Figure 1.0.

Pathology (score)	Lamellar hyperplasia	Lamellar fusion	Cellular anomalies	Lamellar oedema
Index criteria				
None (0)	Not significant – none or very minor	Not significant- none or very minor	none	none
Mild (1)	Mild increase in epithelial cells – low-level of focal or widespread hyperplasia, affecting <10% of gill tissue	Occasional focal fusion of filaments – <10% of gill tissue affected	Scattered occasional degenerating or necrotic cells or focal areas of sloughing	Epithelio-capillary separation with proteinaceous fluid in the epithelia-capillary space: <10% of gill tissue affected
Moderate (2)	Moderate widespread or multifocal hyperplasia, affecting 10-50% if the tissue	Multifocal areas of fusion (moderate widespread hyperplasia affecting 10-50% of gill tissue) interspersed with normal gill tissue	Degenerating cells, necrotic cells and/or cell sloughing in multifocal areas throughout the tissue	10-50% of gill tissue affected
Severe (3)	Extensive lamellar hyperplasia, >50% of gill tissue affected	Extensive fusion and loss of normal architecture, >50% if gill tissue affected	Widespread necrosis, degeneration and/or sloughing visible throughout section	>50% of gill tissue affected
Ancillary criteria— presence or absence of parasites				
Presence (1)				
Absence (0)				

Figure 1.0 Criteria of histopathology scoring of gills adapted from Mitchell *et al* . (2012)

3.5 DATA ANALYSIS

Data was tabulated by using Microsoft Excel and statistical analysis were done by using GraphPad version 9. Shapiro wilk test was used to test the normality of the intensity of

ectoparasites between juvenile and adult fish. Since the data did not pass the normality test, Mann-Whitney test was selected as a non-parametric analysis.

For the histopathology scoring analysis, tabulation of data was done and percentage of fish for each scoring were calculated and presented in pie-chart form.



4.0 RESULTS

4.1 WATER QUALITY TEST

Water quality parameter results are presented in Table 1.0. All of the water quality parameters are normal.

Parameter	Value
pH	7.6
Nitrite	0 ppm
Nitrate	0 ppm
Ammonia	0.5 ppm
Salinity	15 ppt

Table 1.0 Water quality parameters of water sample the sampling location at Sabak Bernam

4.2 PREVALENCE AND INTENSITY OF ECTOPARASITES

In this study, the ectoparasites that were identified were *Trichodina sp.*, *Dactylogyrus sp.*, *Argulus sp.* and *Caligus sp.*

Figure 3.0 shows *Trichodina sp.*, that was found in the skin and gills of *Hexanematchthys sagor* sample. *Trichodina sp.* was observed in all the samples of skin, fin and gills of adult fish. However, none was observed in the skin of the juvenile fish which will be discussed in the discussion section. *Trichodina sp.* can be characterized by the circular or hat-shaped appearance and the circle of hooklets as indicated by red arrow in Figure 3.0.

Similar to *Trichodina sp.*, *Dactylogyrus sp.* were also found in all the samples except for skin scraping sample in both juvenile and adult fish. *Dactylogyrus sp.* can be characterized by the four eye spot indicated Figure 3.2. In the figure, it can also be observed that one *Dactylogyrus sp.* latching on a gill filament.

Two types of crustaceans were found in this study which are *Argulus sp.* (Figure 3.1) and *Caligus sp.* (Figure 3.3). None of the crustaceans were found on both of the skin scraping samples of both juvenile and adult fish while *Caligus sp.*, was only found in adult fish.

The prevalence and intensity of ectoparasites in juvenile and adult fish samples is stated in Table 2.0 and Table 2.1 respectively.

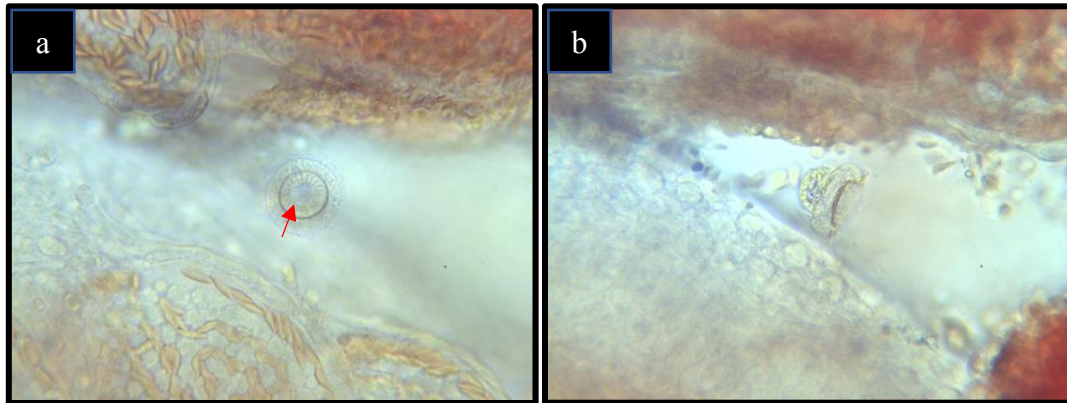


Figure 2.0 *Trichodina* sp. found on a. the skin and b. in the gills of the *Hexanematichthys sagor*



Figure 2.1 *Argulus* sp. found in the gills of *Hexanematichthys sagor*

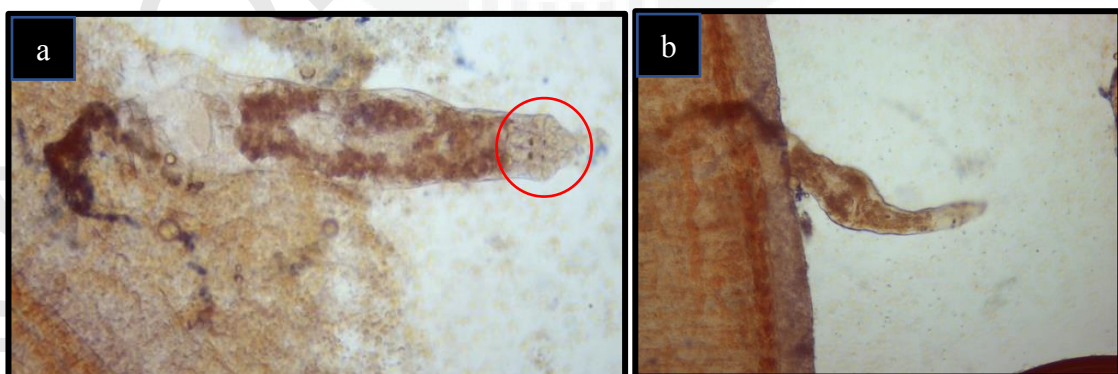


Figure 2.2 *Dactylogyrus* sp. found in the gills of *Hexanematichthys sagor*.
a. *Dactylogyrus* sp. can be distinguished by the four eye-spots indicated by the red circle
b. *Dactylogyrus* sp. latching on a gill filament



Figure 2.3 *Caligus sp.* found in skin of *Hexanematichthys sagor*

Parasites	Total infected fish	Total parasites	Prevalence (%)	Intensity
<i>Trichodina sp.</i>	5	60	100	12
<i>Dactylogyrus sp.</i>	3	22	60	7.3
<i>Argulus sp.</i>	2	7	40	3.5
<i>Caligus sp.</i>	0	0	0	0

Table 2.0 Prevalence and intensity of ectoparasites in Juvenile *Hexanematichthys sagor*

Parasites	Total infected fish	Total parasites	Prevalence (%)	Intensity
<i>Trichodina sp.</i>	5	300	100	60
<i>Dactylogyrus sp.</i>	5	26	100	5.2
<i>Argulus sp.</i>	1	3	20	3
<i>Caligus sp.</i>	1	4	20	4

Table 2.1 Prevalence and intensity of ectoparasites in Adult *Hexanematichthys sagor*

4.2.1 PREVALENCE AND INTENSITY OF ECTOPARASITES BASED ON SAMPLE SITE

Table 3.0 and 3.1 shows the prevalence and intensity of ectoparasites based on the sample sites which are the external surfaces of the fish (skin, fin and gills) of juvenile and adult fish respectively.

Organs	Ectoparasites				Total parasites	Total infected fish	Prevalence (%)	Intensity
	<i>Trichodina sp.</i>	<i>Dactylogyrus sp.</i>	<i>Argulus sp.</i>	<i>Caligus sp.</i>				
Skin	-	-	-	-	0	0	0	0
Gills	30	18	7	-	55	5	100	11
Fin	30	4	0	-	34	5	100	6.8
Total	60	22	7	0				

Table 3.0 Prevalence and intensity of ectoparasites in Juvenile *Hexanematichthys sagor*

Organs	Ectoparasites				Total parasites	Total infected fish	Prevalence (%)	Intensity
	<i>Trichodina sp.</i>	<i>Dactylogyrus sp.</i>	<i>Argulus sp.</i>	<i>Caligus sp.</i>				
Skin	100	-	-	3	103	5	100	20.6
Gills	100	12	3	1	116	5	100	23.2
Fin	100	14	-	-	114	5	100	22.8
Total	300	26	3	4				

Table 3.1 Prevalence and intensity of ectoparasites in Adult *Hexanematichthys sagor*

Based on the tables shown above, it is observed that adult fish have a higher prevalence and intensity of parasites which can also be seen clearer by the bar chart below (Figure 2.0). In skin, intensity of ectoparasites is 0 for juvenile fish and 20.6 for adult. In fin, intensity for juvenile is 6.8 while for adult is 22.8. The gills of both juvenile and adult fish showed the highest parasite intensity of 11 and 23.2 respectively.

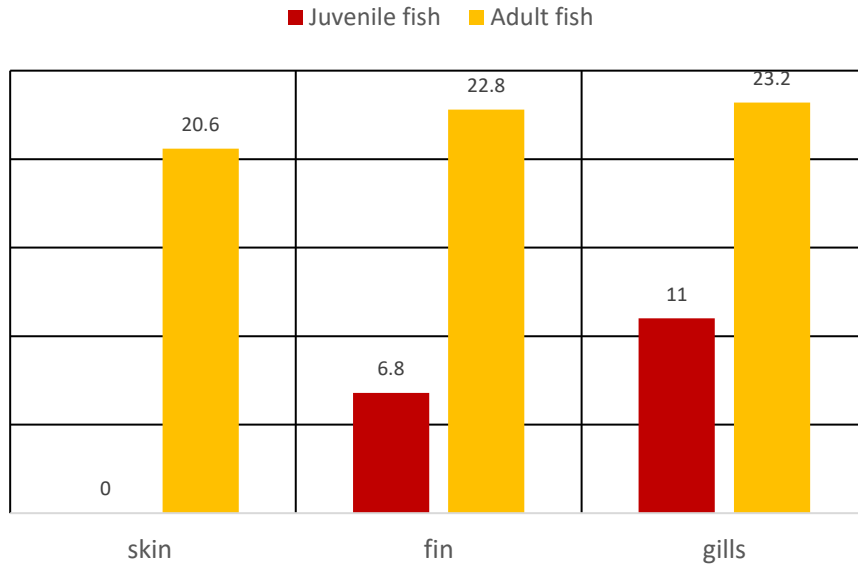


Figure 3.0 The bar chart shows intensity of ectoparasites according to respective site in Juvenile & Adult *Hexanematichthys sagor* sample

Statistical analysis was done through Mann-Whitney test to compare between intensity of ectoparasitic infection in Juvenile and Adult fish and there is no significant difference. Thus, the null hypothesis was accepted.

4.3 HISTOPATHOLOGY ANALYSIS

The histopathology evaluation was done using a microscope under the magnification of 10x and 40x. Each of the histology slides were evaluated and scored using the guideline of gill histopathology scoring as stated in Figure 1.0. For the ancillary criterion, it was evaluated by the presence or absence of ectoparasites. Presence of ectoparasites will be scored as 1 whereas absence of ectoparasites was scored as 0.

4.3.1 HISTOPATHOLOGY RESULT OF JUVENILE FISH

Fish	Lamellar hyperplasia	Lamellar fusion	Cellular anomalies	Lamellar oedema	Ancillary criteria
F1	1	0	0	0	1
F2	1	0	0	0	0
F3	1	0	0	0	0
F4	1	0	0	0	1
F5	1	0	0	0	1

Table 4.0 Histopathology scoring of gills in juvenile *Hexanematichthys sagor*

As stated in Table 4.0, in the juvenile fish samples, it can be observed that all five fish sample were scored 1 for lamellar hyperplasia indicating there are only mild focal hyperplasia that were affecting <10% of the gills. There out of five of the fish scored 1 for the ancillary criterion indicating presence of ectoparasites in the histopathology evaluation.

4.3.2 HISTOPATHOLOGY RESULTS OF ADULT FISH

Fish	Lamellar hyperplasia	Lamellar fusion	Cellular anomalies	Lamellar oedema	Ancillary criteria
F1	2	0	1	0	1
F2	1	0	0	0	1
F3	2	0	0	0	1
F4	2	0	0	0	1
F5	0	0	0	0	1

Table 4.1 Histopathology scoring of gills in adult *Hexanematichthys sagor*

As stated in Table 4.1, in the adult fish samples, three out of five fish sample scored 2 for lamellar hyperplasia, indicating moderate multifocal hyperplasia while one out of five fish was score 1. For cellular anomalies, which was the focal necrosis was only observed in Fish 1 and scored 1. All five fish samples, were scored 1 for ancillary criterion indicating the presence of ectoparasites in the histopathology evaluation.

In Figure 4.0 below, it can be observed that lamellar hyperplasia was the most prominent histopathological lesion in both juvenile and adult fish samples. Although in adult fish, only four out of five fish had a score for lamellar hyperplasia, the scoring is 2 which is

higher compared to juvenile, indicating that the lamellar hyperplasia is more severe in the adults. However, score 2 is still considered as moderate change as described in Figure 1.0.

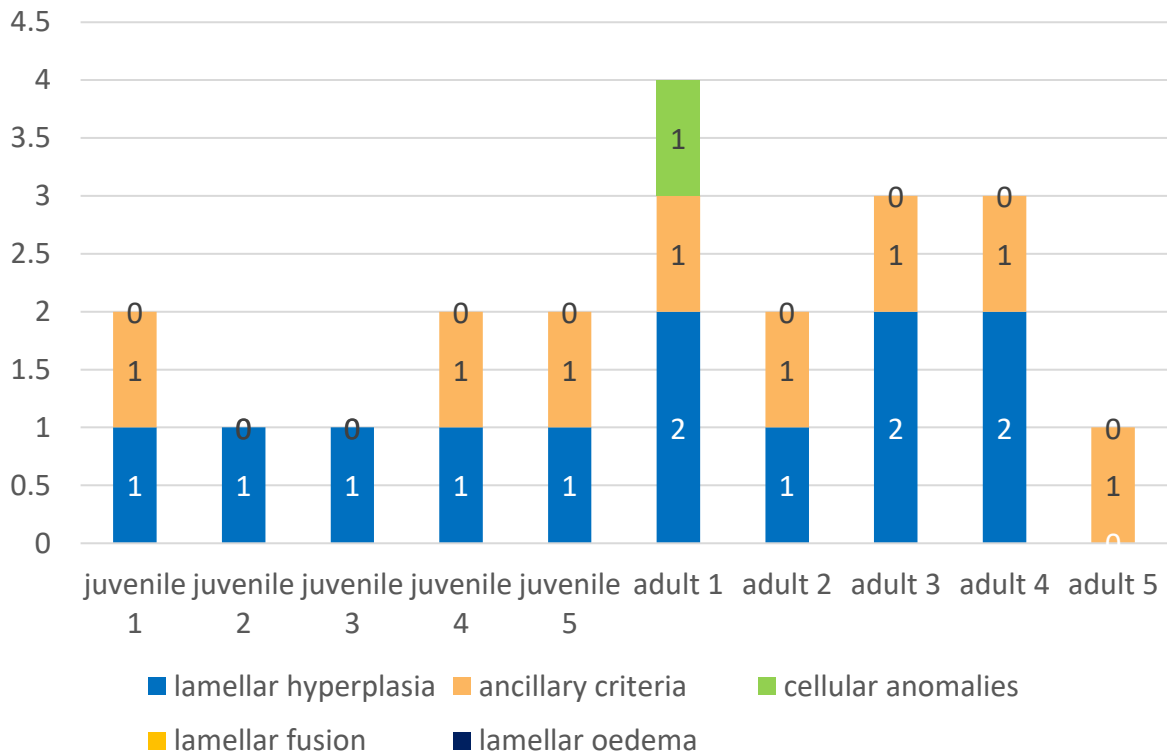


Figure 4.0 Bar chart showing the histopathological scoring of gills for Juvenile & Adult *Hexanematichthys sagor* sample

In Figure 4.1 below, the pie chart shows the combined percentage of lamellar hyperplasia scores in both juvenile and adult fish samples. As stated, 60% of the ten samples were scored 1, 30% scored 2, 10% scored 0 while none scored 3. It can conclude that the lamellar hyperplasia observed in this study were only mild to moderate changes.

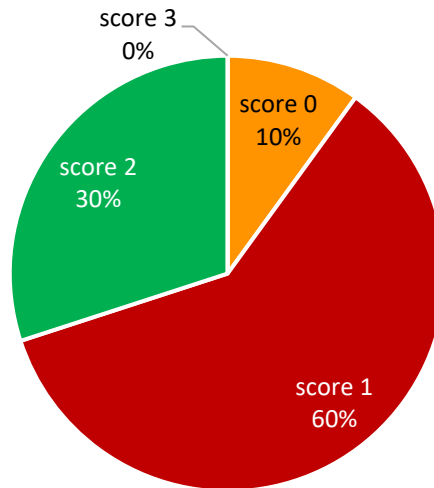


Figure 4.1 Pie chart of percentage of lamellar hyperplasia of gills in Juvenile and Adult *Hexanematichthys sagor* sample

In Figure 4.2 below, the pie chart shows the combined percentage of ancillary criterion scores in both juvenile and adult fish samples. As stated, 80% of the ten samples had presence of parasites observed during the histopathological evaluation while 20% had absence of ectoparasites.

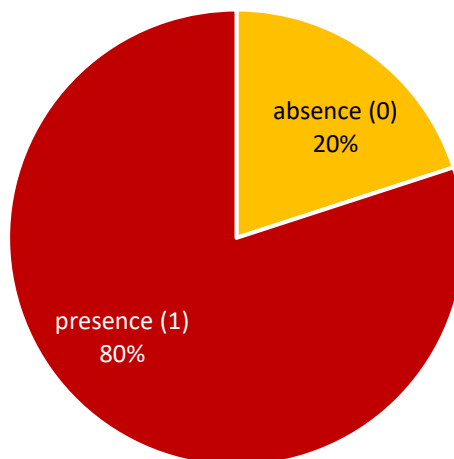


Figure 4.2 Pie chart of percentage of ancillary criterion of gills in Juvenile and Adult *Hexanematichthys sagor* sample

The example of parasites observed during the histopathological evaluation are shown

in Figure 5.0 and 5.1 below. Figure 5.1 shows the ectoparasites that were observed in the gills that were identified as parasites from the digenean group, based from morphological identification. Whereas in Figure 5.1, is the parasitic cyst that were observed in the gills. However, in this study the parasites and parasitic cyst were not further identified until their specific genus or species.

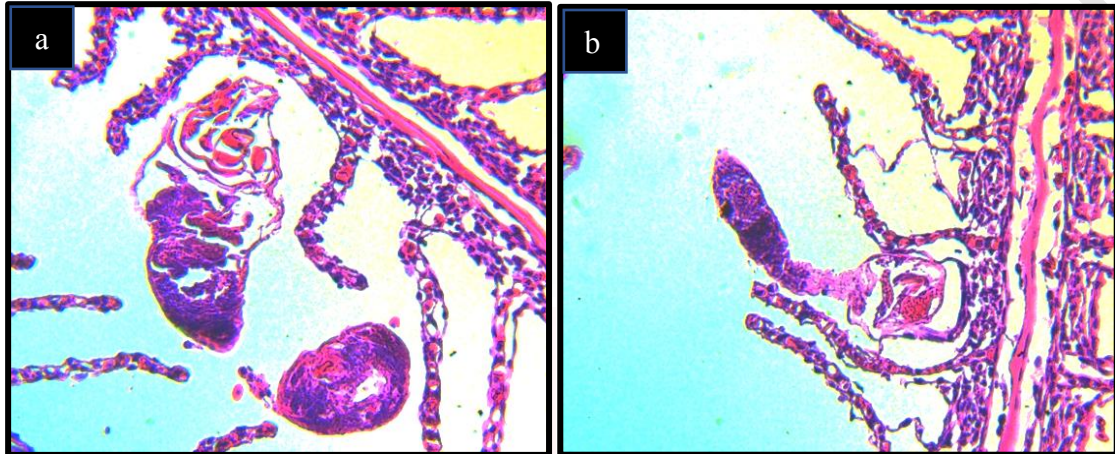


Figure 5.0 Parasites a & b observed during histopathology evaluation of gills of *Hexanematischthys sagor*. The morphology of the parasites under 40x objective lens indicates parasites are from digenean group

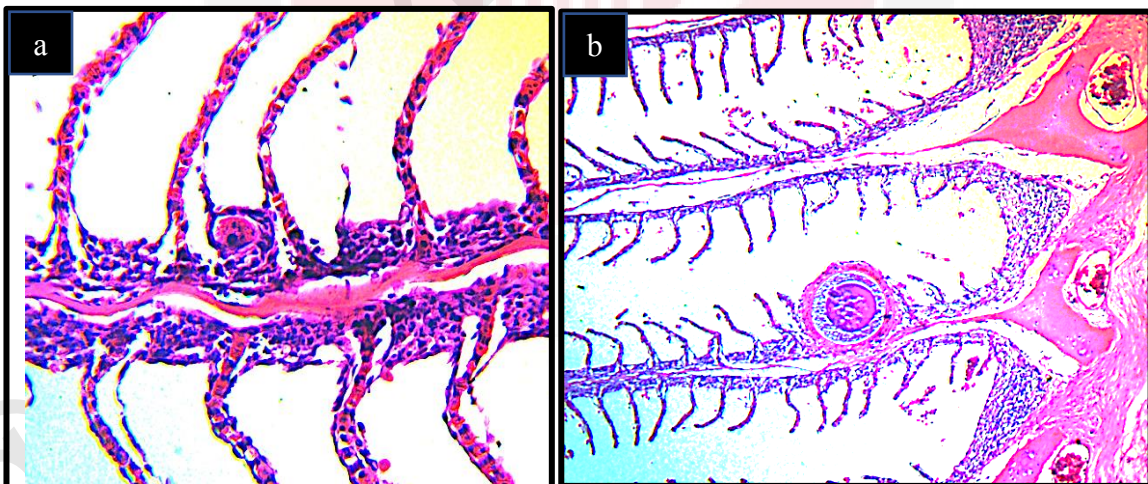


Figure 5.1 Parasitic cyst a & b observed during histopathology evaluation of gills of *Hexanematischthys sagor*

For the cellular anomalies, only one fish sample was observed to have that criteria which had the combination of focal necrosis and lamellar hyperplasia and can be seen in Figure 5.2.

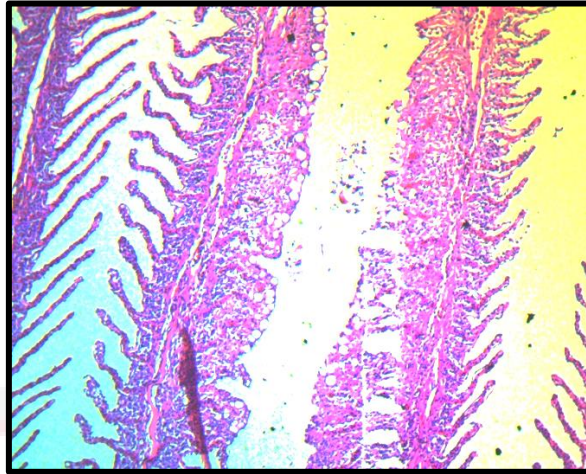


Figure 5.2 Cellular anomalies observed: focal necrosis with lamellar hyperplasia. This histopathology changes were only observed in one adult *Hexanematichthys sagor*

As for other two criteria which are the lamellar fusion and lamellar oedema; none was observed. Statistical analysis was not done as initially histopathological evaluation of the skin and fin were to be done as well. Since the skin and fin sample were not managed to be obtain during this study, statistical analysis was not conducted.

5.0 DISCUSSION

In our current study, the highest intensity of parasitic burden was observed on the gills for both juvenile and adult fish samples. This is because gills have a large surface area and is always in contact with the external environment making it more susceptible (Flores-Lopes & Thomaz, 2011). This study also observed that *Trichodina sp.*, had the highest parasitic prevalence and intensity in both juvenile and adult samples which can be supported by a study stating that *Trichodina sp.* does have preferences towards gills (Gilda et al ., 2001). In the same study, it also stated that the parasite have preference towards the skin. This is consistent with our study where in adult fish samples, *Trichodina sp.*, was found in all ten skin samples. However, this is contradictory with the juvenile samples as none was observed in the samples. This can be due to handling of the fish or contact of the fish among themselves and against the surface of the container that can cause the ectoparasites to die off. *Trichodina sp.* being the most prevalent parasite found in our study is similar to the findings of a study by Kolia *et al* (2021) where the most prevalent parasite found in their fish sample is also *Trichodina sp.* Despite the presence of *Trichodina sp.* found in the skin scraping of the adult fish sample, no lesions were observed through the external visual examination as usually *Trichodina sp.* can feed on the mucus on the skin can lead to lesions and irritations as stated by Smith (2019).

Other than that, current study also shows that adult fish have relatively higher parasitic intensity compared to juvenile as expected. This can be supported from a study that states the high prevalence and intensity might have relation with the attachment area on the fish body, where the bigger fish provides more space for parasites to cling (Muchlisin *et al* ., 2014). As the adults are bigger than juvenile fish it has more space for the ectoparasite to invade. The present study results is also aligned with the study by Eyiseh *et al* (2022) they studied on the incidence of parasitic infection between adult and juvenile of a different catfish species; *Clarias gariepinus*. In their study, it revealed that out of 132 fish sample, 43 adult fish while only 10

juvenile fish were infected with parasite. However, other factors can also influence the parasitic burden that this current study did not take into account for such as sex of the fish sample. In the same study by Eyiseh *et al* (2022), states that adult female fish has higher parasite intensity compared to male fish. Sex determination of our fish samples would help to provide a more accurate representation of our results. Other than that, another factor that can influence the ectoparasite prevalence is the climate change as stated in a study by Kolia *et al* (2021), where the change in water temperature can carry impact to the parasitic infection. However, in our current study, water quality parameters and its association with the parasitic infection of the fish were not investigated.

For the histopathological evaluation of the gills, current studies show that out of the five criteria, lamellar hyperplasia is the most prominent in all of the samples for both juvenile and adult fish samples. If to compare among the four groups of ectoparasite identified in the study, *Trichodina sp.* and *Dactylogyrus sp.* were the most prevalent in juvenile and adults. As stated in a study that *Trichodina sp* and *Dactylogyrus sp.* which is from the monogenean group, can cause lamellar hyperplasia or hyperplasia of the epithelial cells due to the effect of the parasites attaching and feeding on the host's gills (Smith, 2019; Suliman *et al* ., 2021). In a study by Feist & Longshaw (2008) also states lamellar hyperplasia is the initial response and can progress together with lamellar fusion, lamellar stratification and cellular necrosis. However, in the present study only lamellar hyperplasia was observed to be prominent. This can indicate that the samples are responding to an early parasitic infection. The ancillary criterion of presence or absence of ectoparasites in the histopathological evaluation reveals the presence of digenean group parasite as well as parasitic cyst. However, identification was only being able to be done based on morphology of the parasites. Molecular identification can help in identifying the parasites more accurately however it was not done in this current study. This is because depending on morphology identification can cause misinformation and

misinterpretation of the findings due to the different stages in the ectoparasite lifecycle.

The moderate parasitic burden and histopathological changes on the gills are considered normal and not pathogenic towards the wild population of the *Hexanematichthys sagor* in the current study. However, due to the lack of information and studies regarding this species and no report of the ectoparasite occurrence, in depth comparison of the current study results were not able to be done. Improvement and further continuation of this study can be done to help better understand the effect of the ectoparasite occurrence towards this species and any potential risk towards the human consumption and will be discussed further in the recommendations.



@COPY

6.0 CONCLUSION

In conclusion, the ectoparasites that were identified in this study were *Trichodina sp.*, *Dactylogyrus sp.*, *Argulus sp.*, and *Caligus sp.* In all of the 10 samples we obtained, *Trichodina sp.* was observed with the prevalence of 100% and *Dactylogyrus sp.*, was observed in all adult fish. Intensity of ectoparasites was the highest in gills for both juvenile and adult fish samples with 11.0 and 23.2 respectively. As for histopathology evaluation, lamellar hyperplasia was the most prominent with 90% of the gill samples observed and 80% of the samples had the ancillary criterion. This study also revealed that there is no significant difference in the parasitic burden between juvenile and adult fish with p-value >0.05 which is $p= 0.3148$.

7.0 RECOMMENDATIONS

Recommendations for this study is to increase the sample size by increasing the number of fish sample as well as sampling location. This is because by increasing the sample size, a more comprehensive and accurate results can be obtained for the occurrence of ectoparasites of this species in Sabak Bernam, Selangor as the small sample size can influence the parasite prevalence and intensity to be on the higher value. The sampling location can be along the coastline of Sabak Bernam. We can also increase the study period as this can help in obtaining better samples as well as improve study methods. Other than that, is to obtain the skin and fin samples for histopathology regardless of the absence of lesion. This is to achieve the second objective of this study as well as to prove the second null hypothesis. The histopathological changes and evaluation of the skin and fin can add more value in the results of our studies. Another recommendation is to conduct molecular identification by Polymerase Chain Reaction to specifically identify the ectoparasites found on the fish samples to ensure a more accurate determination of the ectoparasite findings of this study. This can help us to evaluate the occurrence of ectoparasites in this species and other similar species in the wild population. Since there is lack of studies of the occurrence of ectoparasites in this species and in wild

population of fish in general, more studies must be done to have better understanding of the ectoparasite impact towards the wild fish. This study can be continued with further improvements.



8.0 REFERENCES

- Afolabi, O.J., Olususi, F.C. & Odeyemi, O.O. Comparative study of African catfish parasites from cultured and natural habitats. *Bull Natl Res Cent* 44, 163 (2020).
- Baharudin, F., Kassim, J., Imran, S.N.M., and Ab Wahab, M. (2021): Water Quality Index (WQI) classification of rivers in agriculture and aquaculture catchments. In *IOP Conference Series: Earth and Environmental Science*. 646(1): 12-23.
- Bichi, A & Dawaki, Salwa. (2010). A survey of ectoparasites on the gills, skin and fins of *Oreochromis Niloticus* at Bagauda fish farm, Kano, Nigeria. *Bayero Journal of Pure and Applied Sciences*.
- Bouwmeester, M. M., Goedknecht, M. A., Poulin, R., and Thielges, D. W. (2021): Collateral diseases: Aquaculture impacts on wildlife infections. *J. Appl. Ecol.* 58(3): 453-464.
- Chong, V. C., Lee, P. K., & Lau, C. M. (2010). Diversity, extinction risk and conservation of Malaysian fishes. *Journal of fish biology*, 76(9), 2009–2066.
- El- Amin Mohamed Suliman, Hassan Ali Osman, Waleed Abdul-Aziz AL Deghayem. (2021). Histopathological changes induced by ectoparasites on gills and skin of *Oreochromis niloticus* (Burchell 1822) in fish ponds. *J App Biol Biotech*. 2021; 9(1): 68-74.
- Falkenberg, J.M., Golzio, J.E.S.A., Pessanha, A. *et al* . Gill parasites of fish and their relation to host and environmental factors in two estuaries in northeastern Brazil. *Aquat Ecol* 53, 109–118 (2019).
- Feist, S.W. and Longshaw, M. 2008. Histopathology of fish parasite infections–

importance for populations. *Journal of Fish Biology*, 73 (9): 2143-2160.

Flores-Lopes, Fábio & Thomaz, Andrea. (2011). Histopathologic alterations observed in fish gills as a tool in environmental monitoring. *Brazilian journal of biology = Revista brasleira de biologia*. 71. 179-88. 10.1590/S1519-69842011000100026.

Fogel D, Fuentes JL, Soto LM, Lafuente W, Moncayo-Estrada R, López C. Ectoparasitic copepod infestation on a wild population of Neotropical catfish *Sciades herzbergii* Bloch, 1794: Histological evidences of lesions on host. *Int J Parasitol Parasites Wildl*. 2017 Oct 6;6(3):344-348. doi: 10.1016/j.ijppaw.2017.10.001. PMID: 29379712; PMCID: PMC5779637.

Froese R. and Pauly, D. (2022): Fishbase electronic publication. www.fishbase.org

Indahsari, M & Kismiyati, Kismiyati & Ulkhaq, Mohammad. (2019). Prevalence and Intensity of Ectoparasites of Tilapia (*Oreochromis niloticus*) in Ponds with Low, Medium and High Stocking Density. *IOP Conference Series: Earth and Environmental Science*. 236. 012108. 10.1088/1755-1315/236/1/012108.

Iriansyah, A. H., Budihardjo, A., and Sugiyarto, S. (2021): Parasites prevalence which infecting freshwater fishes in Mulur Reservoir of Sukoharjo District, Indonesia. *Intl. J. of Bonorowo Wetlands*, 10(2).

Khoo H. (1994). *The Coastal Fisheries of Peninsular Malaysia*.

Klimpel S., Kuhn T., Münster J., Dörge D.D., Klapper R., & Kochmann J. (2019). *Parasites of Marine Fish and Cephalopods*. Springer Nature Switzerland AG.

- McNair, Carol. (2015). Ectoparasites of medical and veterinary importance: Drug resistance and the need for alternative control methods. *Journal of Pharmacy and Pharmacology*. 67.10.1111/jphp.12368.
- Mitchell, Susan & Baxter, Emily & Holland, Celia. (2012). Development of a novel histopathological gill scoring protocol for assessment of gill health during a longitudinal study in marine- farmed Atlantic salmon (*Salmo salar*), *Aquaculture International*, 20, (5), 2012, p813 - 825. *Aquaculture International*. 20. 813-825.
- Mohamed, Suliman & Ali, Osman & Abdul-Aziz, Al-Deghayem. (2021). Histopathological changes induced by ectoparasites on gills and skin of *Oreochromis niloticus* (Burchell 1822) in fish ponds. *Journal of Applied Biology & Biotechnology*. 10.7324/JABB.2021.9109.
- Mohd Fozi, A., Marfiah, A.W., Jazuri, A. and Janmaizatulriah, J. (2015): State of The Coast Report 2012-2014 for Kuala Selangor and Sabak Bernam District. Selangor Waters Management Authority (SWMA). pp. 102-103.
- Muchlisin, Zainal. (2014). Prevalence of ectoparasites on mahseer fish (*Tor tambra Valenciennes, 1842*) from aquaculture ponds and wild population of Nagan Raya District, Indonesia. *Human and Veterinary Medicine*. 6. 148-152.
- Ng, H.H. (2012). *The Ariid Catfishes of Singapore*.
- Smith S. (2019). Dealing with *Trichodina* and *Trichodina*-like species. Virginia Tech, the Virginia Sea Grant College Programme, and Virginia Cooperative Extension.
- Tran Nguyen, Khoa & Mazelan, Suhairi & Muda, Sabri & Shaharom, Faizah.

(2019). The Life Cycle of *Caligus minimus* on Seabass (*Lates calcarifer*) from Floating Cage Culture. *Thalassas*. 35:77-85.

Tukura Echuano Eyiseh, Sogbesan Olukayode Amos, Idowu Thomas Adeniyi. Incidence of parasitic infection in adult and juvenile *Clarias gariepinus* in a private fish farm, Yola, Adamawa state. *Int J Fish Aquat Stud* 2022;10(1):38-45.

Ulhman S. S., Ulrich C., Kennelly S.J. (2019). *The European Landing Obligation*. Springer Nature Switzerland AG.

W S K Kolia, Sunarto & T. Widiyani. (2021). The relationship between water quality and prevalence of ectoparasites on cultured Nile tilapia at two reservoirs in Central Java, Indonesia. *IOP Conf. Ser.: Earth Environ. Sci.* 824 012013