UNIVERSITI PUTRA MALAYSIA

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

INTAN HAZIMAH BINTI ABD.RAZAK

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CERTIFICATIONS

It is hereby certified that we have read this project paper entitled "Occurrence of Endoparasites in Sea Catfish, *Hexanematichthys sagor* (Hamilton, 1822) in Sabak Bernam, Selangor." by Intan Hazimah Binti Abd.Razak and in our opinion it issatisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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This project paper is dedicated to my beloved family, lecturers, staffs, seniors and juniors of Faculty of Veterinary Medicine, UPM and to whom it might be beneficial.

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ABBREVIATIONS

EMC	Encysted Metacercariae Cyst
MMC	Melanomacrophage Centers
PCR	Polymerase Change Reaction
DNA	Deoxyribonucleic Acid

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterianr untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

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

Oleh

Intan Hazimah Binti Abd.Razak

2022

Penyelia: Dr Nur Diyana Binti Mohamad Tahir

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

Hexanematichthys sagor (Hamilton, 1822) atau juga dikenali sebagai ikan keli Sagor ialah ikan keli laut liar yang boleh dimakan dan bukan komersial yang biasa ditemui di Malaysia. Oleh kerana tabiat pemakanannya yang tidak selektif, ikan tersebut mungkin dijangkiti parasit, yang boleh mendatangkan risiko kepada kesihatan orang ramai. Memandangkan jangkitan endoparasit ikan keli ini masih belum didokumenkan di Malaysia, kajian ini bertujuan untuk menilai kejadian jangkitan endoparasit dan perubahan histopatologi dalam ikan keli Sagor juvana dan dewasa. Kajian ini telah dijalankan pada September 2022 di Sekendi, Sabak Bernam. Sepuluh ikan yang terdiri daripada lima juvana dan lima dewasa telah diambil sampel untuk endoparasit semasa nekropsi. Dua puluh lima parasit telah dikumpulkan dari buah pinggang dan usus yang

terdiri daripada genera parasit yang berbeza termasuk nematod, yang mempunyai kelaziman 60% pada ikan dewasa dan 20% dalam juvana, myxozoans, yang mempunyai kelaziman 20% dalam kedua-dua ikan dewasa dan juvana, dan 20% kelaziman metaserkaria encysted yang tidak dikenal pasti (EMC), yang hanya muncul pada juvana. Tiada parasit diperolehi dari hati dan perut. Nematod daripada spesies *Baylisascaris columnaris* telah disahkan melalui PCR konvensional. Ujian Mann-Whitney yang dilakukan menggunakan perisian statik GraphPad Prism v.9.4.1 menunjukkan tiada perbezaan yang signifikan ($p<0.05$) antara ikan keli Sagor dewasa dan juvana dari segi beban endoparasit. Sistem pemarkahan histologi mengenal pasti perubahan struktur dalam buah pinggang, termasuk kehadiran pigmen interstisial (melanomacrophages) dan degenerasi atau nekrosis epitelium tiub. Perubahan seperti penyusupan limfosit dalam lamina propia, rawa epitelium usus dan hiperplasia sel goblet diperhatikan dalam usus. Penemuan kajian ini boleh berfungsi sebagai inventori parasit awal di kawasan itu dan untuk kajian masa depan tentang potensi kesan ke atas penggunaan spesies.

Kata kunci: *Hexanematichthys sagor*; Sabak Bernam; endoparasit; prevalens; histopathologi

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999- Project.

OCCURRENCE OF ENDOPARASITES IN SEA CATFISH, *Hexanematichthys sagor* (Hamilton, 1822) IN SABAK BERNAM, SELANGOR

By

Intan Hazimah Binti Abd.Razak

2022

Supervisor: Dr Nur Diyana Binti Mohamad Tahir

Co-Supervisor: Dr Nor Azlina Binti Abdul Aziz & Dr Mohd Fuad Bin Matori

Hexanematichthys sagor (Hamilton, 1822) or also known as Sagor catfish is an edible, non-commercial wild marine catfish commonly found in Malaysia. Due to its scavenging and non-selective feeding habits, the fish may become infected with parasites, which could pose a risk to the public's health. Since the endoparasite infection of this catfish has not yet been documented in Malaysia, the present study aims to evaluate the occurrence of endoparasite infection and histopathological changes in juvenile and adult Sagor catfish. This study was carried out in September 2022 in Sekendi, Sabak Bernam. Ten fish consisting of five juveniles and five adults were sampled for endoparasites during necropsy. Twenty-five parasites were collected from the kidney and intestine consisting of different parasitic genera including nematodes, which had a prevalence of 60% in

adults and 20% in juvenile, myxozoans, which had a prevalence of 20% in both adults and juvenile, and 20% prevalence of unidentified encysted metacercariae (EMC), which only appeared on juvenile. No parasites were obtained from the liver and stomach. Nematode from the species *Baylisascaris columnaris* was confirmed through conventional PCR. The Mann-Whitney Test performed using GraphPad Prism v.9.4.1 statical software revealed no significant difference ($p<0.05$) between the adult and juvenile Sagor catfish in terms of endoparasites burden. The histological scoring system identified structural changes in the kidney, including presence of the interstitial pigment (melanomacrophages) and tubular epithelial degeneration or necrosis. Changes such as infiltration of lymphocyte in lamina propia, sloughing of intestinal epithelium and goblet cell hyperplasia was observed in the intestine. The findings on this study could serve as a preliminary parasite inventory in the area and for future studies on the potential effects upon consumption of the species.

Keywords: *Hexanematichthys sagor*; Sabak Bernam; endoparasite; prevalence; histopathology

1. INTRODUCTION

1.1 Background

Hexanematichthys sagor (Hamilton, 1822), is a non-commercial species of sea catfish and often referred to as "Duri Pedukang" locally (Froese & Pauly, 2022). This species usually dwells in the coastal habitats like estuaries and mangroves and is occasionally harvested for human use. Fisheries and aquaculture are one of the key economic activities in the seaside town of Sabak Bernam (Baharudin *et al.*, 2021). However, because of the scavenging behaviour of this catfish (Mari, 2022), this catfish species is not as often consumed as other catfish species as there is a lack of parasitic study being carried out on this species. It is currently unclear how growing aquaculture activities may affect collateral diseases especially on the parasitic infection (Bouwmeester *et al*., 2021). As a result, nothing is known about the endoparasite burden in this species. Thus, *Hexanematichthys sagor*, may serve as a sentinel model to fill in the information gaps about the impact of collateral disease on the fish and also consequences of consuming this fish. Wild fish is known to be a definitive, intermediate or parenthetic host for numerous parasite life cycles, including those of protozoan, metazoan, and crustacean parasites (Okulewicz , 2008). All of these parasites could reduce the fish's marketability and cause buyers to worry about the general public's health. Although endoparasite diseases can lower the fish quality and result in financial loss, this species of sea catfish is sold in non-commercial markets (Indahsari., 2019). Additionally, by producing histopathological alterations in the fish tissue where they reside, parasites may negatively impact the health of fish (Timur *et al.*, 2005). Evaluating the histopathological alterations in the organs of parasitized fish may be used as a technique to assess the impact of parasites on fish tissue. The typical effects of parasites on fish include acute or ongoing

inflammation, degenerative alterations, necrosis, and hyperplasia (Feist and Longshaw, 2008).

Studying the parasites that infect fish is important in this setting because they have an impact on both the quality and quantity of the fish. In order to lower the rate of economic losses brought on by parasitic infestations, this study was created to investigate the endoparasite that is common in *Hexanematichthys sagor* fish. The findings in the sea catfish in the sampling sites in Sabak Bernam could potentially help in better understanding on the effect 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.

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1.2 Hypothesis

The hypotheses for this study are:

1H$_0$: There is no significant difference in the burden of endoparasite infection between the juvenile and adult *Hexanematichthys sagor*.

1H$_a$: There is significant difference in the burden of endoparasite infection between the juvenile and adult *Hexanematichthys sagor*.

2H$_0$: Endoparasite infection will not cause significant histopathological changes in the abdominal cavity, gastrointestinal tract and visceral organ of the juvenile and adult *Hexanematichthys sagor*.

2H$_a$: Endoparasite infection will cause significant histopathological changes in the abdominal cavity, gastrointestinal tract and visceral organ of the juvenile and adult *Hexanematichthys sagor*.

1.3 Objectives

The objectives of this study are:

1. To evaluate the occurrence of endoparasites on the abdominal cavity, gastrointestinal tract and visceral organ in juveniles and adult *Hexanematichthys sagor*.

2. To evaluate the histopathological changes caused by endoparasites on the abdominal cavity, gastrointestinal tract and visceral organ in juveniles and adult *Hexanematichthys sagor*.

2 LITERITURE REVIEW

2.1 Sagor Catfish

Figure 1: Sagor catfish (*Hexanematichthys sagor*)
Picture by Heok, H.N.

Hexanematichthys sagor (Hamilton, 1822) family *Ariidae* or also known as the Sagor catfish is a common edible, non-commercial fish species found in Malaysian marine waters. Locally, it is known as "Duri Pedukang" (Froese et al., 2022). Sagor catfish can be easily distinguished from other Arridae it has a combination of broadly rounded, almost spherical supracoccipital spine and a pattern of alternating light and dark vertical bands on the flanks (Ng, 2012). It can be found along the coastline, mainly estuaries (Froese *et al.*, 2022). As this fish is known to be bottom-dwellers and due to its scavenging behaviour of this fish (Mari, 2022), it may serve as intermediate host of various parasites which may debelop into adults in the fish's gut after consumption (Afolabi et al., 2020). The locals used to devour this fish, but an interview with a local fisherman revealed that they no longer do so because of the various parasites that it harbour. Since no research has been done on the parasite infestation of this fish, the information is still remains unclear.

2.2 Sabak Bernam

Sabak Bernam is a coastal town in Selangor, Malaysia with fisheries as one of its main economic activities (Baharudin *et al*., 2021). There are also fish and shrimp farms

located in the area (Hamzah *et al.,* 2009). The wild Sagor catfish is also available in this area.

2.3 Endoparasites of Marine Fish

Endoparasites are parasites that live in the tissue, blood and organs. In wild population, the parasites and the fish host maintain a kind of equilibrium until something happen that disturb the equilibrium balance (Edeh and Solomon, 2017). The wild fish have been reported to have larger parasites species diversity than those raised in hatcheries (Hoffman, 1998). It is expected that the wild marine populations of sea catfish will be carrying multiple species of endoparasites and showing histopathological changes such as inflammatory changes, fibrosis, ulcer and oedema in the abdominal cavity, gastrointestinal tract and visceral organ (Madanire-Moyo *et al.,* 2014) with the burden on adults more compared to the juvenile. It may also cause a drop in the fish body weight (Cone, 2006). With increasing aquaculture activities, the impact of collateral diseases such as endoparasites in wild species is poorly understood (Bouwmeester *et al.,* 2021).

2.4 Parasite of Public Health Concern

Recent global changes have increased the emergence of foodborne parasites in new location and population. Not only that, there is also appearance of new transmission routes which increase the need for more studies and more control measure. Concern for endoparasite infection increases as some of the parasites may show threat to public health as the infected fish is eaten raw (Smith *et al.,* 2002). Fish-borne zoonotic parasites can cause a range of clinical symptoms in human if eaten raw, from mild and transient to chronic, severe, and occasionally life-threatening (Bao *et al.,* 2017). In fact, there are still lack of methods and tools used to detect the presence of the parasites on the food.

3 MATERIALS AND METHODS

3.1 Sample Collection

A total of 10 Sagor catfish (*Hexanematichthys sagor*) were collected for this study. The specimen was obtained from Sabak Bernam, Selangor. The fish was caught using a fish net and some of them were retrieved from the fisherman. The fish were kept in an oxygen-supplied container along the necropsy process.

3.2 Morphometric Measurements

The body morphometric measurements were done according to the method describe by Mohamed (2003). The total length (TL), and standard length (SL) were measured to the nearest 0.1cm.

3.3 Examination of the Specimen

The fish was examined directly for the presence of parasites at the collection sites under IACUC approval (UPM/IACUC/AUP-U005/2022). Fish was handled cautiously using a damp cloth to reduce stress and injuries to the skin surface and to prevent the skin from drying out. The fish were then immobilized by decapitation and pithing of the central nervous system of the fish prior to further examination. The endoparasite was examined by dissection of the fish internal organs for direct observation under the light microscope. The dissection was done by piercing the abdominal cavity using a pair of scissors at the ventral midline just behind and between the pectoral fins and cutting along the ventral midline towards the anal region. The abdominal flap was removed to expose the internal organs of the abdominal cavity. The stomach, intestines, liver and kidney were carefully taken out and put on a petri dish. Any white spots found on the surface of the organs was

carefully examined to check on the possibilities of a parasite infestation. Crush examination was done on the liver and kidney and were observed using a stereomicroscope. The intestine was incised into 3 different portions namely the anterior, middle and posterior part and was put on a different petri dish for easier identification and examination of the entire gastrointestinal tract. The 3 parts of the intestine was dissected carefully, and the intestinal walls were scrapped. The scrapings were placed onto a clear glass slide and one drop of methylene blue was placed on the slide. These were carefully examined under a microscope for the attachment of the parasites using 10x and 40x magnification.

3.4 Histology Sample Processing & Scoring

For histological studies, the target organs (stomach, intestine, liver and kidney) were carefully removed and fixed in 10% Buffered Formalin for 24 hours. Tissues were dehydrated and processed at Histopathology Laboratory (FPV) for paraffin wax embedding. The sections were cut by a rotary microtome and stained with hematoxylin and eosin. Tissues were examined for histopathological lesion under light microscope. Scoring was done according to the fish organ histopathological changes scoring suggested by Jovanovic *et al.*, (2014).

3.5 Parasite Identification

Nematode isolated during necropsy was further identified through semi-permanent mounting technique using clove oil. Conventional PCR was done for species identification using NucleoSpin® Tissue DNA Purification Kit by using Cox1 gene. The forward and reverse primers used were LCO1490 and HCO2198 respectively.

3.6 Statistical Analysis

The prevalence of parasites was estimated through the suggested formula (Margolis *et al.,* 1982). The parasitic data observed were analyzed using Mann-Whitney Test performed using GraphPad Prism v.9.4.1 statical software to observe the significant difference ($p<0.05$) between the adult and juvenile Sagor catfish in terms of endoparasites prevalence. Microsoft Excel was used for data tabulation.

Parasites Prevalence = No of infected hosts ÷ Total no. of host examined x 100

4 RESULTS

4.1 Prevalence of Endoparasites

The prevalence calculation was done manually according to the total number of parasites that was observed. The below tables show the prevalence of endoparasites found on the adult (Table 1) and juvenile (Table 2) of *Hexanematichthys sagor* catfish from Sabak Bernam, Selangor.

Common Parasites	Adult			Prevalence	Organ
	No. of Fish Examined	No. of Fish Infected	No. of Parasite		
Cestode (Tapeworms)	5	0	0	0.00%	-
Trematodes (Flukes)	5	0	0	0.00%	-
Nematodes (Roundworms)	5	3	10	60.00%	Intestine
Acanthocephala (Thorny-headed worms)	5	0	0	0.00%	-
Myxozoan	5	1	3	20.00%	Kidney
Unidentified Encysted Metacercariae (EMC)	5	0	0	0.00%	-

Table 1: Prevalence of Endoparasites in Adult Sagor catfish from Sabak Bernam, Selangor

Common Parasites	Juvenile			Prevalence	Organ
	No. of Fish Examined	No. of Fish Infected	No. of Parasites		
Cestode (Tapeworms)	5	0	0	0.00%	-
Trematodes (Flukes)	5	0	0	0.00%	-
Nematodes (Roundworms)	5	1	5	20.00%	Intestine
Acanthocephala (Thorny-headed worms)	5	0	0	0.00%	-
Myxozoan	5	1	6	20.00%	Kidney
Unidentified Encysted Metacercariae (EMC)	5	1	1	20.00%	Kidney

Table 2: Prevalence of Endoparasites in Juvenile Sagor catfish from Sabak Bernam, Selangor

Five *Hexanematichthys sagor* for each juvenile and adult, were used in total for the prevalence study. A total of 25 parasites were found in the kidney and intestine, belonging to various parasitic genera. During the necropsy process, Nematode parasites from an unknown species were encountered inside the intestine of the fish (Figure 2C). For further species identification, the Nematode sample was forwarded to the Parasitology Laboratory of the Faculty of Veterinary Medicine, UPM. The presence of the Myxozoan (Figure 2A) and Unidentified Encysted Metacercariae (UEM) (Figure 2B) were observed inside the kidney from the histology slides prepared.

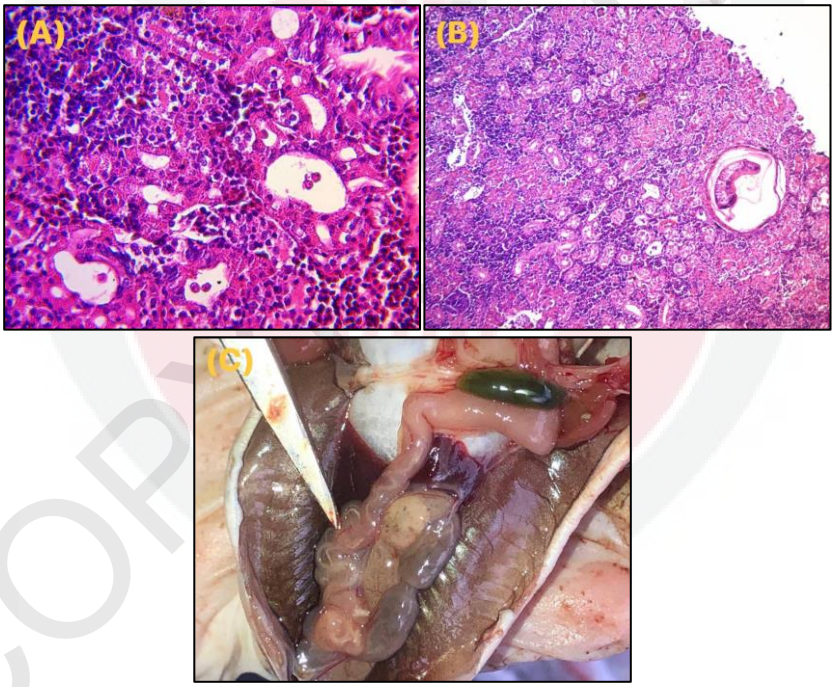

Figure 2: Presence of Myxozoan (A), Unidentified Encysted Metacercariae (B) and Nematode (C) Inside the Intestine and Kidney of *Hexanematichthys sagor*.

According to the endoparasites prevalence result of the adult (Table 1) and juvenile (Table 2) *Hexanematichthys sagor*, Nematodes were the most common, with a prevalence of 60% in adults and 20% in juveniles. Myxozoans were the second most common, with a prevalence of 20% in both adults and juveniles. Unidentified encysted

metacercariae (EMC), which only affected juveniles, had a 20% prevalence. The liver and stomach were examined however, no parasites were found. The adult and juvenile Sagor catfish had the same endoparasite load, and there is no significant difference ($p<0.05$) between the adult and juvenile Sagor catfish in terms of endoparasites burden according to the Mann-Whitney Test run using GraphPad Prism v.9.4.1 statical software.

4.2 Histopathological Changes

The organ histopathological changes scoring was done according to the scoring guide suggested by Jovanovic *et al*., (2014). The scoring was done using a scale which consist of none (0), mild (1), moderate (2) and severe (3). The changes were noticed on the kidney and intestine of the fish. No histopathological changes were observed from the liver and stomach organ.

Histopathological Changes	Adult					Juvenile				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Congestion	0	0	0	0	0	0	0	0	0	0
Tubular epithelial degeneration/necrosis	2	0	1	2	0	0	2	0	0	0
Interstitial pigment (melanomacrophages)	3	0	1	0	0	0	1	0	0	0
Presence of interstitial hematopoietic tissue	0	0	0	0	0	0	0	0	0	0

Table 3: Histopathological Changes Scoring for Kidney of the Sampled Sagor catfish

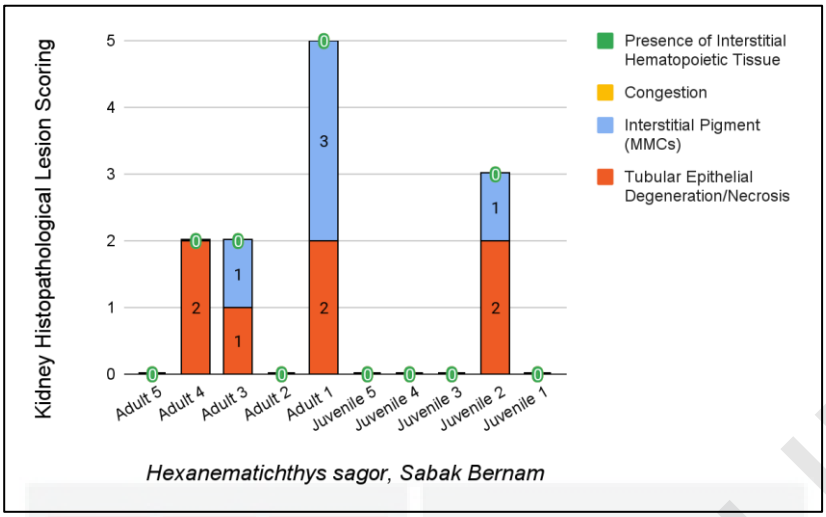

Figure 3: Kidney Histopathological Changes Scoring of *Hexanematichthys sagor*, Sabak Bernam

The histopathological changes in the kidney of each fish were tabulated (Table 3). According to the kidney histopathological changes scoring result (Figure 3), tubular epithelial degeneration/necrosis was observed with a score of none (1) to moderate (2) in the adult fish and score of none (0) and moderate (2) in the juvenile. The presence of kidney interstitial pigment (melanomacrophages) in the adult fish scored from none (0) to severe (3), while the juvenile scored none (0) to mild (1). All fish scored none (0) for the congestion and the presence of interstitial hematopoietic tissue.

Histopathological Changes	Adult					Juvenile				
	1	2	3	4	5	1	2	3	4	5
Infiltration Of Lymphocyte In Lamina Propria	1	0	1	1	0	0	0	0	0	1
Sloughing Of Intestinal Epithelium	1	0	1	1	0	0	0	0	0	2
Goblet Cell Hyperplasia	0	0	1	2	0	0	0	0	0	0

Table 4: Histopathological Changes Scoring for Intestine of the Sampled Sagor Catfish

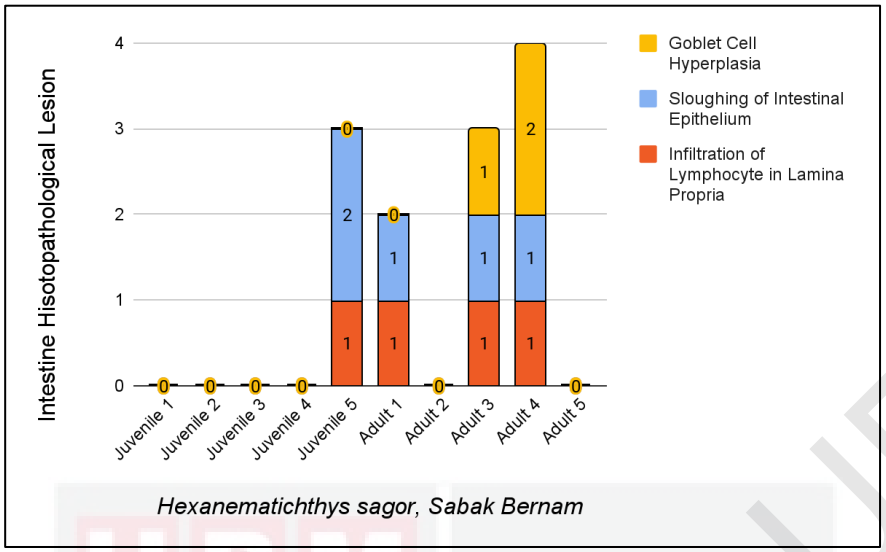

Figure 4: Intestine Histopathological Changes Scoring of *Hexanematichthys sagor*, Sabak Bernam

The histopathological changes in the intestine of each fish were also documented (Table 4). According to the intestine histopathological changes scoring result (Figure 4), goblet cell hyperplasia was only noticed in the adult fish with a score of none (0) to moderate (2). Sloughing of the intestinal epithelium was appeared to be none (0) and moderate (2) in the juvenile, while the adult recorded a score of none (0) to mild (1). The infiltration of lymphocytes in the lamina propria of both, the adult and juvenile fish scored none (0) to mild (1).

4.3 Parasite Species Identifications

Nematode samples from the species *Baylisascaris columnaris* was confirmed through the conventional PCR and semi-permanent mounting. The PCR products yields and amplification of 710 base pairs fragments which was similar to the *Baylisascaris columnaris* as suggested by Choi *et al.,* (2017). The accession no. was KY580739 and this nematode is 87.42% identical to the one found in the Salt Lake County, US.

4.4 Water Quality Analysis

The water quality analysis shows that all parameters as listed below (Table 5) for the sea water at Sabak Bernam were within the range and is suitable for the growth of the Sagor catfish based on the recommendation by FAO (2010).

Parameter	Sampling site at Sabak Bernam, Selangor
pH	7
Ammonia	0.5ppm
Nitrite	0ppm
Nitrate	0ppm

Table 5: Water Quality Analysis of Sabak Bernam, Selangor

5 DISCUSSIONS

Our initial research indicates that there is no significant difference between the adult and juvenile *Hexanematichthys sagor* in the burden of endoparasite infection. In this study, adult fish (Table 1) recorded a greater prevalence of nematode infection than the juvenile fish (Table 2) with 60% and 20% respectively. This result was comparable to that of Ahmad *et al.,* (2021) study's, in which adult fish specimens were found to be heavily infected, followed by sub-adult fish species, while no infection was seen in juvenile fish species. Paggi *et al.,* (1982) stated that heavy infection with nematode in fish can cause haemorrhagic and inflammation, but it may not necessarily always distrupt fish growth. According to research done by Omeji *et al.,* (2014), parasite infection rises with length and weight. However, this research runs counter to Akinsanya *et al.,* (2007) study's, which claims that the gastrointestinal parasite infections in juvenile specimens are more susceptible to parasitic infections than adult specimens. The difference in the parasite prevalence results can be due to various factors such as the parasite species and their biology, host and its feeding habits, other physical factors, hygiene of the body water and also presence of the intermediate hosts (Doreen *et al*., 2009). The prevalence of parasites in the natural environment are also largely dependent on other factors such as the geographic range, diet, local habitat use and size of the host fish

The tubular epithelial degeneration/necrosis and presence of interstitial pigment (melanomacrophages) which scored none (0) to severe (3) are the histological alterations that occurred in the kidney (Figure 3) in this study. The severe tubular epithelial degeneration/necrosis of the kidney was comparable to the findings of Maftuch *et al*., (2018) investigation's, which showed that *Myxobolus sp.* infected Koi carp exhibit apparent kidney cell destruction and necrosis. This is further confirmed by the findings

of Watchariya *et al*., (2012) who discovered that myxosporidiosis, which affects the kidney, results in cast-like formation and degeneration of the tubular epithelium cells.

The intestinal histopathological changes (Figure 4) ranged from none (0) to moderate (2) which include the goblet cell hyperplasia, intestinal epithelium sloughing, and lymphocyte infiltration in the lamina propria. Thatcher (1991) and Molnar *et al.*, (1994) suggested that nematode parasites may be responsible for the atrophy in the intestinal villi during its feeding phase. Findings by Salah Eldeen *et al*., (2012) showing the total loss of intestinal villi infected with nematode is characterised by damage to intestinal villi and leukocyte infiltration further support these alterations.

It was determined that the nematode obtained was of the *Baylisascaris columnaris* species which came from the genus *Ascarididae*. The fact that this species descended from the same *Baylisascaris procyonis* species that appears to be zoonotic, it raises questions about its potential impact on public health. Ingestion of embryonated *Baylisascaris sp*. eggs orally has been linked to visceral, ophthalmic, and neurological larva migrans, according to a study by Franssen *et al*., (2013). However, this study also suggests that compared to *B. procyonis*, *B. columnaris* may be less harmful. However, because there is currently a dearth of research and publications on *B. columnaris*, appraisal of its relevance to public health is still unclear.

6 CONCLUSION

In conclusion, the study mentioned above found that there is no significant difference in the load of endoparasite infection between adult and juvenile *Hexanematichthys sagor*. The histopathological alterations in the fish's organs ranged in severity from none (0) to severe (3). The *Baylisascaris columnaris* nematode species' existence in the fish is another sign of a possible zoonotic transmission, raising questions on the public health concern.

7 RECOMMENDATIONS

Recommendations that can be suggested for further study would be that the study period should be extended to a longer period which include both the rainy and dry seasons in order to properly assess parasite invasion. In order to observe the parasite differences in the prevalence, the fish sample should be taken from a variety of location sites or states. Aside from that, I would advise including hemoparasite in the research to accurately assess the parasite's harmful effects on the host. Moreover, larger sample sizes can also be applied to reduce statistical error. Last but not least, I would advise adding other organs to cover the potential endoparasite infestation sites, such as the muscle and spleen.

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