



UNIVERSITI PUTRA MALAYSIA

**SCREENING ON WILD BLUE GOURAMI (*Trichogaster trichopterus*)
FOR MYCOBACTERIOSIS, EPIZOOTIC ULCERATIVE SYNDROME,
WHITE SPOT DISEASE AND TREMATODE INFECTION IN TWO
POPULATIONS WITHIN KLANG, SELANGOR**

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

2023/2024

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**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
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.**

December 2023

CERTIFICATION

It is hereby certified that I have read this project paper entitled “Screening On Wild Blue Gourami (*Trichogaster trichopterus*) For Mycobacteriosis, Epizootic Ulcerative Syndrome, White Spot Disease And Trematode Infection In Two Populations Within Klang, Selangor”, by Asma Fariha binti Asma Halim, 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 – Final Year Project.

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DEDICATION

This project paper is devoted to Allah S.W.T., the Creator who shaped my existence and facilitated every aspect of this project.

To my family,

My father, Asma Halim bin Harith,

My mother, Fazilah binti Rahman,

My sibling; Asma Hamzah bin Asma Halim,

My friends,

And to all my mentors and teachers who have dedicated themselves towards the noble cause of education. I express my heartfelt gratitude for your unwavering support and care.

May this serve as a source of inspiration and motivation for your future pursuits.

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acid sulfate soils

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
EUS	Epizootic ulcerative syndrome
sp	Species
±	Plus minus
x	Times
g	Gram
ml	Mililiter
RM	Ringgit Malaysia
pH	Potential of hydrogen

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian keperluan kursus VPD4999 – Projek Tahun Akhir

Pemeriksaan Ke Atas Ikan Gurami Biru Liar (*Trichogaster trichopterus*) bagi Mikobakteriosis, Sindrom Ulseratif Epizootik, Penyakit Bintik Putih, dan Jangkitan Trematoda di Dua Populasi di Klang, Selangor

Oleh

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2023

Penyelia: Prof. Madya Dr Md Sabri Mohd Yusof

Penyelia bersama: Prof. Dr Mohd Fuad Matori

ABSTRAK

Ikan gurami biru (*Trichogaster trichopterus*) mempunyai nilai hiasan dan nilai kuliner yang bernilai tinggi bagi negara. Pengesanan penyakit pada spesies ini boleh memberi impak yang signifikan terhadap industri perikanan Malaysia. Walaupun ikan ini rentan kepada pelbagai penyakit seperti mikobakteriosis, sindrom ulseratif epizootik, penyakit bintik putih, dan jangkitan trematoda, kajian terhadap kadar prevalens penyakit ini di kalangan ikan gurami biru liar masih terhad, terutamanya di Klang, kawasan industri di Selangor, Malaysia. Tujuan kajian ini adalah untuk mengisi kesenjangan penyelidikan ini dengan menilai kadar prevalens keempat-empat penyakit ini di kalangan ikan gurami biru liar di dua lokasi berbeza di Klang serta menilai perbezaan prevalens antara populasi tersebut. Kajian ini tidak hanya menyumbang kepada pemahaman yang

lebih baik tentang status kesihatan populasi ikan gurami biru liar, tetapi juga meletakkan asas untuk penyelidikan dan kajian masa depan. Dua puluh ekor ikan gurami biru dewasa telah ditangkap dari dua lokasi yang berbeza dan diperiksa untuk mengesan kehadiran lesi khas. Ikan yang terjangkit dicatat, dan kadar prevalens diukur. Secara mengejutkan, kajian ini mendedahkan hasil yang tidak dijangka: tiada satu pun dari ikan yang diperiksa terjangkit mikobakteriosis, sindrom ulseratif epizootik, atau penyakit bintik putih. Hanya satu ekor ikan (0.05%) yang mengalami jangkitan trematoda. Trematoda digenean yang ditemui dicurigai adalah *Clinostomum* spp., sejenis parasit zoonotik yang tersebar melalui ikan dan boleh menyebabkan laringitis dan faringitis dalam populasi manusia. Tiada perbezaan prevalens di antara kedua-dua populasi. Secara keseluruhan, prevalens penyakit keempat-empat ini dalam kalangan ikan gurami biru liar adalah sifar hingga minimum, yang mungkin disebabkan oleh beberapa faktor. Walau bagaimanapun, masih tidak jelas sama ada penyakit-penyakit ini benar-benar jarang berlaku dalam kalangan ikan gurami biru liar. Ini menyarankan perlunya penyelidikan yang lebih mendalam melibatkan populasi yang lebih besar untuk mendapatkan pandangan yang bernilai tentang dinamika kesihatan ikan gurami biru liar.

Kata kunci: Ikan Gurami Biru, Mikobakteriosis, Sindrom Ulseratif Epizootik, Penyakit Bintik Putih, Jangkitan Trematoda, Klang

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD4999 – Final Year Project

**Screening On Wild Blue Gouramis (*Trichogaster Trichopterus*) For
Mycobacteriosis, Epizootic Ulcerative Syndrome, White Spot Disease And
Trematode Infection In Two Populations In Klang, Selangor**

By

Asma Fariha binti Asma Halim

2023

Supervisor: Assoc. Prof. Dr Md Sabri Mohd Yusof

Co-Supervisor: Dr. Mohd Fuad Matori

Blue gourami (*Trichogaster trichopterus*) has valuable ornamental and culinary value for the country. Detection of disease in this species could have had a significant impact on the Malaysian fishing industry. Despite its susceptibility to various diseases such as mycobacteriosis, epizootic ulcerative syndrome, white spot disease, and trematode infection, limited research has been conducted on the prevalence rates of these diseases in wild blue gourami, especially in Klang, an industrial area in Selangor, Malaysia. The aim of the study was to fill this research gap by assessing the prevalence rates of the four diseases in wild blue gourami at two different locations in Klang and evaluating the difference in

prevalence between the populations. This study not only contributes to a better understanding of the health status of wild blue gourami populations, but also lays the foundation for future research and studies. Twenty adult wild blue gouramis were captured from two different locations and examined for the presence of characteristic lesions. Infected fish were recorded, and the prevalence rate was measured. Surprisingly, the study revealed an unexpected result: none of the fish examined were infected with mycobacteriosis, epizootic ulcerative syndrome, or white spot disease. Only one fish (0.05%) had trematode infection. The digenean trematode found was suspected to be *Clinostomum spp.*, a fish-borne zoonotic parasite that causes laryngitis and pharyngitis in the human population. There was no difference in prevalence between the two populations. In summary, the overall prevalence of the four diseases in wild blue gourami is zero to minimal, which could be due to several factors. However, it remains unclear whether these diseases are genuinely rare in wild blue gourami. This recommends further in-depth research involving larger populations to gain valuable insights into the health dynamics of wild blue gourami.

Keywords: *Blue gourami, mycobacteriosis, epizootic ulcerative syndrome, white spot disease, trematode infection, Klang*

1.0 INTRODUCTION

1.1 Background

Blue gourami (*Trichogaster trichopterus*), a member of the Anabantidae family, is a species of freshwater labyrinth fish, known for its ornamental and culinary value. It is distributed throughout most of southern Asia (Forselius, 1957; Hodges et al., 1964; Vierke, 1988). Because of its labyrinth organ, a specialized breathing organ, it is often found in areas with low oxygen content, such as rice fields and swampy regions. Referred to by locals as *ikan sepat padi*, it was once a culinary staple in suburban areas. Also, due to its adaptability and easily maintainable ornamental fish, it is famous among fish enthusiasts. This species fetches a retail price of RM3-5 in the Malaysian market and Anabantidae fishes contributed approximately RM81 thousand to the total export ornamental value (Department of Fisheries, 2022). This dual significance underscores the cultural and economic relevance of the Blue gourami.

However, Blue gourami is susceptible to various diseases. This includes Epizootic ulcerative syndrome, an OIE-listed fungal disease, caused by *Aphanomyces invadans* (OIE, 2013). The fungus is highly invasive and proliferative, causing high mortality and morbidity to the wild and farmed fish population. Since its outbreak in 1972, it has become a major issue across the globe. There have been no reported cases of EUS in Malaysia since the 1980 outbreak, in Bekok River, Johor. However, in 2023, the Aquatic Animal Health

Unit reported the emergence of new cases of EUS in the northern region of Peninsular Malaysia.

Similarly, Blue gourami is susceptible to white spot disease or ichthyophthirius, the most common protozoan disease in freshwater fishes, caused by a ciliate protozoan, known as *Ichthyophthirius multifiliis*. It causes severe epizootics among various fish species in aquaria, hatcheries, and ponds, as well as in wild fish populations (Ezz El-Dien et al., 1998; Thilakaratne et al., 2003; Kim et al., 2002). But in Malaysia, there has been no study conducted to demonstrate the occurrence and prevalence of White spot disease in Blue gourami.

Blue gourami is also susceptible to mycobacteriosis, one of the most common bacterial diseases of aquarium fish (Astrofsky et al., 2000). The infected fish are frequently associated with gross lesions ranging from skin ulceration to tumourlike nodules in the visceral organs, such as liver, kidney and spleen (Hashish et al., 2018). Apart from fish tuberculosis, mycobacteria are capable of causing both localized and disseminated infections in man (Lewis et al., 2003).

Another zoonotic disease that Blue gourami susceptible to is trematode infection. It is caused by digenetic trematodes, parasitic flatworms that are categorized as liver, lung, intestinal or gill flukes (Kieu et al., 2019). Digeneans are usually asymptomatic infections that mainly inhabit the gastrointestinal tract (Noga, 2010). It is reported that the consumption of raw fish is the main pathway that affects the health of more than 40 million people across the globe. In Malaysia, there is no updated prevalence status of zoonotic trematode infection.

Justification

Blue gourami are susceptible to these four diseases. However, the actual distribution and prevalence of these diseases in Malaysia is still not well-known. There is not much study to demonstrate the prevalence of these diseases. Since these diseases have been reported to have serious public health implications, it is important to detect the presence of these diseases in the wild population of Blue gourami and raise public awareness.

Objective of study

The objective of this study was to determine the prevalence of Epizootic ulcerative syndrome, Mycobacteriosis, White spot disease and trematode infection in wild Blue gourami and to assess the difference in prevalence between the two populations.

Hypothesis of study

H₀₁: There will be no detection of Mycobacteriosis, Epizootic ulcerative syndrome, White spot disease and Trematode infection in wild Blue gourami (*T. trichopterus*) in Klang, Selangor.

H_{A1}: There will be detection of Mycobacteriosis, Epizootic ulcerative syndrome, White spot disease and Trematode infection in wild Blue gourami (*T. trichopterus*) in Klang, Selangor.

H₀₂: There will be no difference in the prevalence of Mycobacteriosis, Epizootic ulcerative syndrome, White spot disease and Trematode infection between the two populations of wild Blue gourami (*T. trichopterus*) in Klang, Selangor.

H_{A2}: There will be difference in the prevalence of Mycobacteriosis, Epizootic ulcerative syndrome, White spot disease and Trematode infection between the two populations of wild Blue gourami (*T. trichopterus*) in Klang, Selangor.

2.0 Literature review

2.1 Distribution of wild Blue gourami

The Blue gourami (*Trichogaster trichopterus*) is a labyrinth fish that belongs to the Anabantidae family in the Anabantiformes order (Van Der Laan et al., 2014). There are 16 discovered genera comprising 80 species, distributed vastly across southern Asia, India and central Africa (Figure 1) (Degani, 2001; Forselius, 1975; Vierke, 1988). Like many other labyrinth fish, Blue gourami is equipped with a specialized air-filled breathing cavity that enhances its breathing mechanism. Situated above the gills beneath the operculum, on top of the head behind the eyes, this labyrinth is characterized by highly dense wrinkled tissue, increasing the surface area available for gaseous exchange (Degani, 2011). Due to this unique function, Blue gourami is able to survive in low oxygen content and is commonly found in water bodies with such conditions. Their habitat includes rice paddy fields, swamps and ponds with rich vegetation.

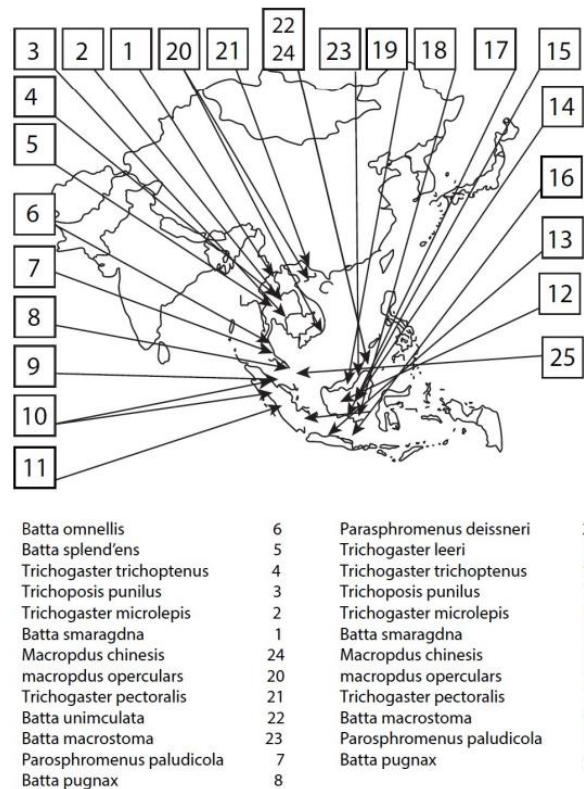


Figure 1: Distribution of labyrinth fish in Southeast Asia (Degani 2001; Vierke 1988)

2.2 Food preference of Blue gourami

In Malaysia, the Blue gourami is known locally as *ikan sepat padi*. Especially in suburban regions where paddy fields are prevalent, this fish stands as one of the most commonly consumed freshwater fish. Based on the fish consumption pattern among adults of different ethnics in Peninsular Malaysia, out of 10 in every 100 adults prefer gourami as their food choice over the other 12 types of freshwater fishes (Figure 2). Among local communities, the common methods of cooking fish are deep frying, constituting 75% of the total, and cooking it in a thick chili gravy (*masak sambal*), which accounts for 19%

(Ahmad, N. I., et al., 2016). Locals find them easy to catch, and this accessibility has possibly become the reason for their selection as a preferred food.

Given these factors, Blue gourami holds a notable demand in the local market, with a relatively expensive price ranging from RM3 to RM5 for a batch of 8-10 fish. Up to this day, there have been no scientific studies conducted to demonstrate the market value of this species, but the presence of online trade within the export market sheds some light on its demand and potential value.

Freshwater fish				
1	Keli	Freshwater catfish	<i>Clarias batrachus</i>	77
2	Haruan	Snakehead fish	<i>Channa micropeltes</i>	31
3	Sepat	Gourami	<i>Trichogaster trichopterus, Trichopodus pectoralis</i>	19
4	Patin	Iridescent shark	<i>Pangasius hypophthalmus</i>	17
5	Talapia	Tilapia	<i>Oreochromis mossambicus</i>	13
6	Puyu	Climbing perch	<i>Anabas testudineus</i>	9
7	Sebarau	Hampala	<i>Hampala macrolepidota</i>	7
8	Kelah	Mahseer	<i>Tor tambroides</i>	6
9	Jelawat	Hoven's carp	<i>Leptobarbus hoevenii</i>	3
10	Lampam	Java barb	<i>Barbonymus gonionotus, B. schwanenfeldii</i>	2
11	Baug	Bagrid catfishes	<i>Chrysichthys nigrodigitatus</i>	2
12	Pacu	Pacu	<i>Piaractus mesopotamicus</i>	1
Cephalopods and mollusk				
1	Udang	Prawn and shrimp	<i>Penaeus monodon, P. semisulcatus, M. affinis, P. japonicus, Parapenaeopsis sculptilis, M. ensis, Metapenaeus brevicornis, M. barbata, Parapenaeopsis hardwickii, P. merguensis, P. indicus, Parapenaeopsis hardwickii, P. latisulcatus</i>	298
2	Sotong	Squid and octopus	<i>Loligo uyii, Cistopus indicus, L. chinensis, L. duvauceli, L. edulis, L. sibogue, Sepia esculenta, S. phuruonis</i>	263

^aSpecies names were based on fish landed and/or available at the wholesale market in Peninsular Malaysia (26-28) and at the website: www.fishbase.org/ComNames/CommonNameSearchList.

^bThe frequency of fish obtained from the subjects of the food consumption survey (3-day records). Of the fish consumption records, 40% did not mention fish name, and therefore are not included in this list.

Figure 2: Fish consumption pattern among adults of different ethnics in Peninsular Malaysia (Ahmad, N. I., et al., 2016)

2.3 Market value as ornamental fish

According to recent reports from the Department of Fisheries, Malaysia is the eighth-largest producer of ornamental fish in the world, and more than 70% of the species produced are exported. The major groups of ornamental fish

produced in Malaysia consist of freshwater species from the family of Anabantidae, Callichthyidae, Characidae, Cichlidae, Cyprinidae, Cyprinodontidae, Loricariidae, Osteoglossidae, and Poeciliidae (Department of Fisheries Malaysia, 2019). Among these families, Anabantidae has contributed approximately RM22,000 to the total ornamental export value (Figure 3). Holding the second position after Singapore, Malaysia contributes 9% to global trade (Othman M. F, et al., 2017). Among other freshwater fish species, the Blue gourami (*Trichopodus trichopterus*) serves as one of the important species for ornamental fish trade in Malaysia.

According to the European Ornamental Fish Import and Exports, Malaysia recorded an income of \$1,133 million exclusively through ornamental fish exportation to the EU countries. Other countries that are involved in the export markets include Hong Kong, Japan and Taiwan. The growing industry of ornamental fish exportation and importation has led to disease outbreaks, which are highlighted as one of the main issues faced by aquaculture farms. This issue has become a chronic problem for the past 20 years. Diseases reported in most farms are of bacterial and viral origin. Apart from that, emerging global diseases have also been reported in several farms. These disease outbreaks cause huge economic losses.

PENGELUARAN DAN NILAI IKAN HIASAN, 2019
Production and Value of Ornamental Fish, 2019

IKAN HIASAN Ornamental Fish	2018		2019		PERUBAHAN / Changes (%)	
	Kuantiti (Ekor) Quantity (Pieces)	Nilai Value (RM '000)	Kuantiti (Ekor) Quantity (Pieces)	Nilai Value (RM '000)	Kuantiti Quantity	Nilai Value
Cyprinids	100,103,414	112,097.51	99,653,669	98,058.43	-0.45	-12.52
Anabantids	19,694,038	24,137.80	22,645,770	39,818.66	14.99	64.96
Poecilids	45,243,216	13,047.37	44,601,416	22,048.18	-1.42	68.99
Characins	27,595,078	23,865.73	21,515,668	20,114.76	-22.03	-15.72
Chichlids	9,191,730	13,358.60	11,091,199	138,786.31	20.66	938.93
Osteoglossids	765,873	107,794.34	1,046,800	139,370.32	36.68	29.29
Callichthyids	46,860,027	44,474.38	200	1.50	-100.00	-100.00
Cobitids	-	-	-	-	-	-
Loricariidae	13,087,508	3,037.02	6,160,689	1,233.08	-52.93	-59.40
Lain-lain (Others)	62,787,619	8,513.39	80,816,128	47,016.56	28.71	452.27
JUMLAH BESAR Grand Total	325,328,503	350,326.14	287,531,539	506,447.79	-11.62	44.56

Figure 3: Production and value of ornamental fish in Malaysia (Department of Fisheries Malaysia, 2019)

2.4 Mycobacteriosis

Mycobacteriosis is an important and most common bacterial disease of fishes. It is caused by mycobacterium species that are gram-positive, nonmotile, pleomorphic bacilli with strong acid-fast staining properties. According to Novotny et al. (2004), *Mycobacterium marinum*, *M. fortuitum* and *M. chelonae* are among the most identified species causing fish mycobacteriosis. This disease has been known to infect tropical pet fish leading to a high morbidity and mortality rate (Chang and Whipps, 2015). Astrofsky et al. 2000 mentioned that freshwater fishes belonging to the families Anabantidae, Characidae and Cyprinidae are most susceptible to infection with Mycobacterium.

The infected fish are frequently associated with various symptoms, such as abdominal swelling, emaciation and uncoordinated swimming. Gross lesions

typically range from skin ulceration to tumorlike nodules at the visceral organs. These nodules are characterized by small, creamy whitish miliary nodular structures, and the organs that are most commonly affected are the spleen, kidney and liver (Hashish et al., 2018).

Mycobacteriosis is easily transmissible via exposure or direct inoculation of the organism through wounds or any opening. It takes around weeks or months to fully become apparent. During this incubation period, the health of the fish deteriorates, leading to emaciation and uncoordinated swimming (M. Pate et al., 2005). A decreased growth rate or increased mortality are common findings. Some fish able to survive after years being infected with chronic mycobacteriosis. Granulomatous lesions in the visceral organs are classic findings during postmortem (Spickler Anna Rovid & Dvorak Glenda, 2020).

2.4.1 Zoonotic potential

Mycobacterium sp. has been recorded to have the potential to cause zoonotic infections in humans. Mycobacteriosis, also known as "the fish handler's disease," is frequently found in people working in seafood industry and keeping fish as a hobby. Infections in humans majorly involve skin, particularly in areas that have come in contact with contaminated water. This includes distal extremities such as knee, elbow and foot. Mycobacteriosis in humans typically

has skin lesions characterized by localized papulopustular lesions that may develop into clusters (Spickler Anna Rovid & Dvorak Glenda, 2020). However, in recent years, there have been fewer cases reported on this zoonotic disease, possibly due to the developing awareness and education among people about the disease. Even in Malaysia, mycobacteriosis outbreak has yet to be reported despite being highlighted as a common bacterial disease in shrimp farms (Hashish et al., 2018).

2.5 Epizootic Ulcerative syndrome

Epizootic ulcerative syndrome (EUS) is an OIE-listed, seasonal and dangerous disease in freshwater fishes. It causes a significant impact on the wild and farmed fish population, damaging the livelihood of fishermen. Since its outbreak in 1972, it has become a major issue across the globe. The causative agent of EUS is an aquatic fungus called *Aphanomyces invadans*, which belongs to the family Saprolegniaecea. The fungus is highly invasive and proliferative, and an infection of less than 10 zoospores can cause extensive infection across multiple organs, leading to mortality of the fish. Typical manifestations of lesions include severe dermal and muscle ulceration. Early EUS lesions are said to be caused by erythematous dermatitis with no obvious oomycete involvement.

According to the OIE Manual of Diagnostic Tests for Aquatic Animals 2021, *Trichogaster trichopterus* is one of the fish species susceptible to be naturally infected by *A. invadans* (OIE, 2013).

Since its first outbreak in Japan in 1971, the disease has spread to multiple countries including Australia, Singapore, (Roberts et al., 1989), Bangladesh, Malaysia (Lilley et al., 1998), and many other countries. In 2007, a confirmed outbreak of EUS was identified in Botswana, Namibia and Zambia (FAO, 2009). Up until now, EUS outbreaks have been reported from more than 20 countries in four continents, which are North America, Southern Africa, Asia and Australia (Figure 3). There have been no reported cases of EUS in Malaysia since the 1980 outbreak, in Bekok River, Johor. However, recently reported emergence of new cases in the northern region of Peninsular Malaysia are underway for publication.



Figure 4: Map showing the geographical spread of EUS in the last three decades
(K. R. John, & M. R. George, 2012)

2.6 Trematode infection

Digenetic trematodes are a group of parasitic flatworms that affect all vertebrates, including fish and human. According to the typical microhabitat in which the adult parasite typically resides, they are categorized as liver, lung, intestinal, or gill flukes (Kieu et al., 2019). Digeneans are usually asymptomatic infections that mainly inhabit the gastrointestinal tract (Noga, 2010). The transmission mechanism of this agent to fishes and human is typically similar for

all species; the trematode eggs are ingested by the intermediate host (i.e. snail), followed by a free-swimming cercariae encysted stage that sticks to the skin or any part of the host fish. Based on Tawewan T. et al. (2014), the infection of trematode in *Trichogaster spp.* is one of the most significant problems associated with pond fish culture in Thailand.

2.6.1 Zoonotic potential

Some species are zoonotic to humans, causing liver (clonorchiasis, opisthorchiasis) and intestinal (heterophydiasis and fasciolopsiasis) infections. Due to the involvement of fish as intermediate or definitive hosts of trematodes, it become a potential source of infection to human population. It is reported that the consumption of raw fish is the main pathway that affects the health of more than 40 million people across the globe. Fish-borne trematode infections have a worldwide distribution, with Southeast Asia and Western Pacific Regions being particularly prevalent (Cromie, R.L. et al., 2012).

In Malaysia, there is no updated prevalence status of trematode infection in aquaculture sector. In 2005, the first case of fasciolopsis (intestinal fluke) was reported in a 39-year-old female farmer, a native of Sabah. The finding was incidental, but it entails the importance of further investigation of actual

distribution and level of infection of this long-forgotten disease (Institute for Medical Research, Malaysia - Home, 2023).

2.7 White spot disease

White spot disease or Ichthyophthirius, is the most common protozoan disease in freshwater fishes. It is caused by a ciliate protozoan, known as *Ichthyophthirius multifiliis*, that has a direct lifecycle with no intermediate hosts; trophont, tomite, theronts. The trophont infiltrates and encysts between the skin and gill epithelium of the fish host. This gives the appearance of white blisters on the affected area (Buchmann, 2019). Under 40x magnification, the agent is identified by the appearance of a large, oval structure with a horseshoe-shaped macronucleus (Figure 5) (Dickerson, H. W., 2012). The parasite is widely distributed, with populations found in tropical, subtropical, and temperate regions as well as north to the Arctic Circle (Matthews, 1994; Elsayed et al., 2006). It causes severe epizootics among various fish species in aquaria, hatcheries, and ponds, as well as in wild fish populations (Ezz El-Dien et al., 1998; Thilakaratne et al., 2003; Kim et al., 2002).

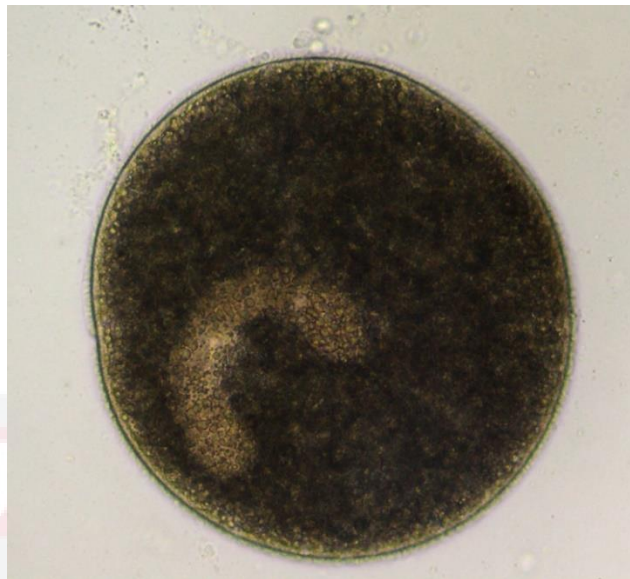


Figure 5: *Ichthyophthirius multifiliis* (Dickerson, H. W., 2012)

In Malaysia, white spot is a common disease infecting mainly juveniles of freshwater fishes. In 2012, ichthyophthirius was detected on the gills of Malaysian mahseer (*Tor tambroides*), terbul fish (*Puntius gonionotus*) and baung fish (*Mystus nemurus*) at Lake Kenyir, Terengganu (Faizah, S. 2012). Recently, in 2022, 46.42% of the Peacock Bass specimens were found infected by *I. multifiliis* at Tasik Telabak, Terengganu, Malaysia (Aina Sabihah et al., 2023).

Up until now, there has been no study conducted to demonstrate the occurrence and prevalence of White spot disease in Blue gourami.

3.0 METHODOLOGY

3.1 IACUC Approval

This study was conducted upon approval on the IACUC reference code UPM/IACUC/AUP-U006/2023.

3.2 Sample collection

20 adult Blue gourami fish were collected using casting nets from marshes in Kapar, a town in Klang, Selangor. Two groups of ten adults were caught in two different locations: Location A ($3^{\circ}12'29.847''\text{N}$ $101^{\circ}24'1.817''\text{E}$) and Location B ($3^{\circ}12'15.614''\text{N}$ $101^{\circ}24'44.938''\text{E}$). The fish were then transported to Universiti Putra Malaysia's Aquatic Animal Health Unit (AAHU) for processing. All of the fish were euthanized humanely using the pithing technique. Any presence of clinical manifestations was recorded. This includes abdominal distension, exophthalmia, emaciation, or any other abnormalities.

3.3 In-situ water quality

The water quality of the swamp area was determined via a YSI probe meter. Important characteristics that were taken are temperature, pH, salinity, and

dissolved oxygen.

3.4 Fungal examination (Epizootic ulcerative syndrome)

The fish were examined for gross lesions. Those exhibiting ulcerative lesions or red spots on their body surfaces were selected for skin scraping and stained with Grocott's stain. Meanwhile fish that do not exhibit the typical lesions were selected for histopathology sample. All slides were stained with hematoxylin and eosin (H&E). The presence of the agent, which is characterized by basophilic hyphae surrounded by granulomas, was recorded. Any histological changes within the muscle tissue were also recorded.

3.5 Ectoparasitic examination (White spot disease)

The fish were examined for gross lesions with white spots. Wet mount sampling from the body surface, fins, and excised gills was done. Skin scrapings were obtained using the blunt side of scalpel blade #20 which the mucous was spread onto a glass slide to be examined under the light microscope. The gills were then observed for any lesions. Gills that were affected with white spot lesions were taken to be examined microscopically (Frimeth, 1994; Noor-Shahirah et al., 2018). The presence of the agent, which has the distinctive shape

of a round body shaped with a large crescent-shaped nucleus, was identified and recorded.

3.6 Endoparasitic examination (Trematode infection)

Wet mount sampling from the excised gills was done. The gills were exposed and examined for the presence of metacercariae. The affected portion of the gill was excised using a pair of iris scissors and placed in a drop of saline water on a glass slide to be examined under a light microscope.

The fish then were dissected to examine the visceral organs. Any presence of metacercariae was identified and recorded. A squash smeared was done. The muscular tissue from the abdominal cavity was cut using iris scissor. The tissue were placed between two glass slides and squeezed gently with fingers. The tissue then covered with a cover slip and viewed under a light microscope.

3.7 Bacterial examination (Mycobacteriosis)

All fish were dissected to expose the abdominal organs. The internal organs, such as liver, spleen and kidney were examined for any presence of granulomatous lesions. These lesions are characterized by small and cream-white

miliary nodule-like structures. Organs displaying these lesions were chosen for further examination. Smears made directly from the affected organs were stained with Ziehl-Neelsen acid-fast stain before being observed under the light microscope. Meanwhile, fish that did not exhibit typical lesions were selected for histopathology samples to detect the presence of microgranuloma.

3.8 Data analysis

3.8.1 Prevalence of disease

The prevalence of diseases was calculated using the following formula (Noor-Shahirah et al.,2018):

$$\left(\frac{\text{No. of diseased fish}}{\text{No. of sample population}} \right) \times 100$$

3.8.2 Statistical analysis

Statistical analyses were carried out to determine the significance of association of prevalence between two populations of wild Blue gourami. Fisher's exact test was used with significant value ($p < 0.05$) and confidence interval of 95%.

4.0 RESULT

4.1 Gross lesions

In the present study, a total of 20 fish were examined for presence of typical lesions on the surface of body and visceral organs. 20 fish showed no significant gross lesions on body surface, 19 fish showed no significant lesions on visceral organs (Figure 6) and 1 fish showed presence of metacercariae at viscera (Figure 7).

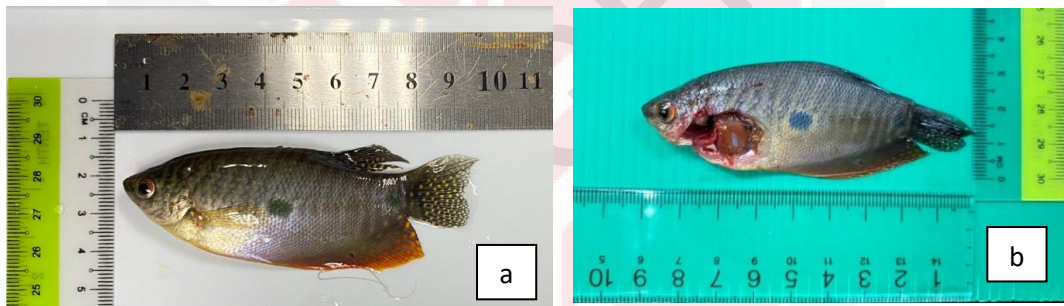


Figure 6: Photographs of uninfected wild Blue gourami. On body surface (a) and visceral organs (b).



Figure 7: Presence of metacercariae observed at the visceral organs (red circle).

4.2 Smears findings

Skin scrapings, gill smears and squash smears from 20 fish were taken for analysis to detect presence of *Ichthyophthirius multifiliis* and trematodes. All smears revealed no presence of agents (Figure 8).

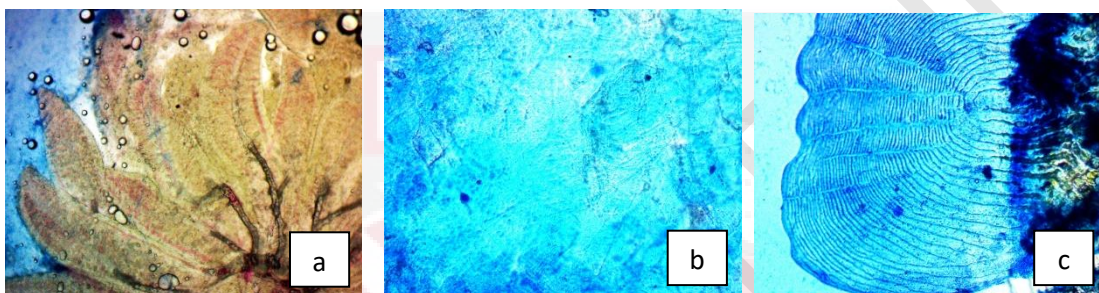


Figure 8: Smears taken from uninfected fish. Gill sampling (a), squash smears (b) and skin scrapings (c).

4.3 Prevalence of diseases

The prevalence of EUS, Mycobacteriosis, White spot disease and trematode infection is presented in Table 1. No specimens examined tested positive for EUS, Mycobacteriosis, and White spot disease. This makes the prevalence of these three diseases zero. Meanwhile, the prevalence of trematode infection showed only 0.05% (1/20), which gained from Location A.

Location	A	B
Epizootic ulcerative syndrome	0	0
Mycobacteriosis	0	0
White spot disease	0	0
Trematode infection	0.05%	0

Table 1: Prevalence of EUS, Mycobacteriosis, White spot disease and Trematode infection in Klang, Selangor

4.4 Correlation of prevalence between two populations

Fisher's exact test, applied to the disease prevalence data from the two populations, yielded $p > 0.05$. These results suggest that there is no significant association between the disease prevalence and the population origin.

4.5 Histopathology findings

As stated in the methodology, fish that do not exhibit typical lesions of Epizootic Ulcerative Syndrome (EUS) and Mycobacteriosis were selected for histopathological analysis. Tissue specimens, including the liver, spleen, kidney, and intestine, were extracted from 20 fish. However, all the samples revealed no presence of agent of interest (*Aphanomyces invadans* & *Mycobacterium spp.*)

However, most of the tissue samples (liver, spleen and kidney) showed a high saturation of iron and presence of melanomacrophages centres (Figure 9).

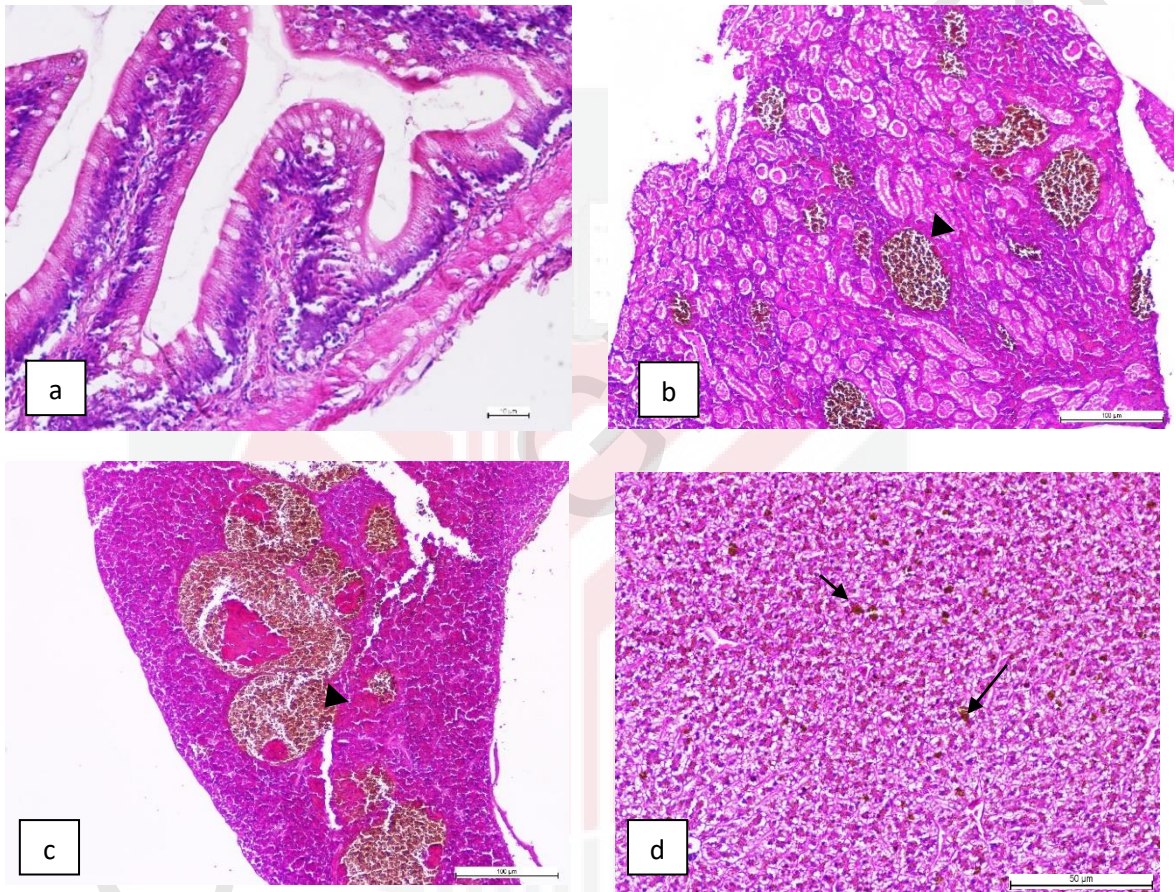


Figure 9: Histopathology of uninfected fish. Intestines (a), spleen (b), kidney (c) and liver (d). Melanomacrophages in spleen and kidney (arrowhead) and iron saturation in liver parenchyma (arrow). H&E stain, a = x40, b,c = x10, d = x20, scale bar 10 μm.

4.6 Parasitological findings

Out of 20 fish, one fish was infected with trematode. Presence of trematodes was identified in the abdominal cavity, indicated by whitish, dorsoventral flattened body (Figure 10). Based on morphological characteristics, the trematodes could belong to *Clinostomum spp.* (Hirotsuka Hara et al. 2014).



Figure 10: Trematodes (*Clinostomum spp.*) in the abdominal cavity (arrow)

5.0 DISCUSSION

The result of disease prevalence and correlation between the two populations of wild Blue gourami showed no significant difference. This showed a good sign that the wild population of Blue gourami is not affected by the four important and common diseases; EUS, mycobacteriosis, white spot disease or trematode infection. However, it is inevitable that environmental conditions could be the main factors influencing this finding.

It is observed that the water samples collected during sampling were moderately acidic; Location A: 5.6 and Location B:3.7. The low soil pH might be because they are naturally acidic, or disturbed by agricultural or residential development. Either might lead to a lowering of soil and water pH (Baldock et al., 2005; Choonga et al., 2009). The pH value of water samples plays an important role in the survivability of pathogens. According to Wagner's research in 1960, *Ichthyothirius multifiliis* can only survive within a pH range of 5.5 to 10.1, while mycobacterium species require a pH level higher than 5.8 for their growth. Because the pH levels from the sampling site were outside the suitable range for these pathogens, it could possibly suggest that the agents were not present in the water samples collected during this study. Apart from that, according to Oidtman 2012, a pH range of 6-7 could be one of the factors that influence the expression of EUS. Because the pH values recorded at my sampling site, were below the optimal range, it suggests that the expression of EUS was potentially hindered during my sampling.

Another possible factor that could contribute to this is the seasonal pattern. Seasonal pattern comprises of wet and dry seasons, that is characterized by the frequency of precipitation. In other words, the wet season is a period where most of the region's average annual rainfall occurs while the dry season experiences little to no rainfall. Certain causative agents thrive differently in response to different seasonal pattern. According to the Malaysian Meteorological Department (2020), the dry season in Malaysia occurs between May and September, and the sampling was done in August, precisely during the

dry season. *Aphanomyces invadans* is known to sporulate favourably in response to heavy rainfall, as documented by Lumanlan Mayo (1997). Given this characteristic, it could suggest that the climatic condition during the sampling period might not have been conducive to the presence of this agent in the collected water samples.

Aside from that, the zero prevalence of these diseases could be due to the possibility that the infected fish may have succumbed to mortality or fallen prey to other predators, including larger fish or birds. This is because infected fish may exhibit abnormal behavior, such as uncoordinated swimming patterns, making them more vulnerable targets for predators. For example, fishes that are infected with EUS will stop feeding, come to the surface, and remain vertical (Devashish K., 2014). Natural predators in the ecosystem might be controlling the population of diseased fish. According to Hatcher et al., 2006, predators eat infected prey, sometimes quite preferentially. This reduces the chances of disease spread by decreasing contact between susceptible and infected hosts. Consequently, it reduces host density, making less diseased fish detectable in the population of the sampling area. However, further study on the actual densities of natural predators in that area should be conducted to gain a better understanding of their role in disease prevention.

In this study, the prevalence of trematode infection is considerably low, at 0.05%. This contrasts sharply with the finding in Kedah, showing a remarkably high prevalence which is 70.5% (43/61) (Rahman and Bakri, 2008). The huge difference in prevalence could be because of different sampling sites. The study

conducted in Kedah was done at paddy fields while the sampling area in this study was conducted at an irrigation canal. Paddy fields are subjected to the usage of pesticides, such as herbicides (Fuad et al., 2012). These pollutants may flow into the aquatic environment, affecting the health and immune response of fish. In turn, the fish become more susceptible to diseases. This may explain why the prevalence of trematodes was remarkably high in Kedah compared to this study.

One of 20 fish was infected with trematode infection. Based on the morphological characteristics (Hirota Hara et al. 2014), the trematode species could belong to *Clinostomum spp.*, a zoonotic trematode species (Ulrich Sinsch et al., 2021). According to Lee et al. (2017), ingesting metacercariae of *Clinostomum spp.* could cause acute pharyngitis or laryngitis. The prevalence of *Clinostomum spp.* in Malaysia is still unknown, but a study conducted in neighbouring country, Sumatera, Indonesia showed a significantly high prevalence of *Clinostomum spp.*, in wild Blue gourami, which is 93.9% (31/34). Due to the high proximity of Malaysia and Indonesia, which also shares similar climatic conditions, it could suggest that the prevalence of *Clinostomum spp.* in Malaysia is high too. However, the actual distribution and prevalence of this parasite still remains unknown, hence further investigation on this should be considered.

Histopathology findings of tissue samples revealed a remarkably high saturation of iron and presence of melanomacrophages. The sampling site of this study, which is Klang located along the coastal area of Peninsular Malaysia. According to Shamshuddin Jusop (2017), acid sulphate soil typically distributed along the coastal region (Figure 11). This soil is characterized by low acidity, high iron and aluminium content. This may explain increase uptake of iron in the soft tissue of the samples observed in this study.



Figure 11: A map of Peninsular Malaysia showing the distribution of acid sulphate soils (shaded area)

6.0 CONCLUSION

In conclusion, 20 wild Blue gourami in Klang, Selangor were not infected with Epizootic ulcerative syndrome, Mycobacteriosis, White spot disease, and only 1 fish was infected with trematode infections. There was no correlation of prevalence of these diseases between the two populations. Histopathology findings showed no presence of agents but revealed high saturation of iron and presence of melanomacrophages despite fish showing no clinical signs. The environmental factors could be main factors that contribute to this finding but many other possible factors that should be explored as well.

7.0 RECOMMENDATION

For further study, a larger sample size and longer duration of study can be conducted to extend the epidemiology data and understand better the prevalence of these diseases in different populations of wild Blue gourami. In addition, molecular study of these diseases can be done for rapid and accurate diagnosis by using molecular tests, such as Polymerase Chain Reaction (PCR). Also, a prospective study can be conducted to gain better understanding of disease progression in wild population of Blue gourami.

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