UNIVERSITI PUTRA MALAYSIA

GROSS AND HISTOPATHOLOGICAL CHARACTERISTICS OF ANABAS TESTUDINEUS ACQUIRED FROM PEAT SWAMP AND FEEDER CANAL OF PEAT SWAMP FOREST IN NORTH SELANGOR, MALAYSIA

NURNAJWA ATIQAH BINTI MD SALLEHUDDIN

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A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia Serdang, Selangor Darul Ehsan.

2020/2021

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Gross and Histopathological Characteristics of *Anabas testudineus* acquired from Peat Swamp and Feeder Canal of Peat Swamp Forest in North Selangor, Malaysia", by Nurnajwa Atiqah Binti Md Sallehuddin and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4901 - Project.

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DEDICATION

I dedicate my dissertation work to my family and many friends.

A special feeling of gratitude to my loving mother, Siti Hajar Binti Mohd Idris whose words of encouragement and push for tenacity ring in my ears.

My father and my two brothers which have never left my side and are very special.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful.

All praises to Allah and His blessing for the completion of this thesis. I thank God for all the opportunities, trials and strength that have been showered on me to finish writing the thesis. I experienced so much during this process, not only from the academic aspect but also from the aspect of personality. My humblest gratitude to the holy Prophet Muhammad (Peace be upon him) whose way of life has been a continuous guidance for me.

First and foremost, I would like to sincerely thank my supervisor Dr Mazlina Mazlan for her guidance, understanding, patience and most importantly, she has provided positive encouragement and a warm spirit to finish this thesis. It has been a great pleasure and honour to have her as my supervisor.

Futhermore, I would also like to thank both my co-supervisors, Dr. Mohd Fuad Matori and Dr Awang Hazmi Awang Junaidi for their ideas, enlightenment, knowledge and experiences in coming up with this project and helping me to improve it along the way.

My deepest gratitude goes to all of my family members. It would not be possible to write this thesis without the support from them. I would like to thank my dearest mother Siti Hajar for helping me throughout the process of finishing my fyp project.

I would sincerely like to thank all my beloved friends who were with me and support me through thick and thin. Most importantly I would like to thank staff of the Veterinary Histopathology Laboratory, Pn. Jamilah, Pn. Latifah, Cik Aisyah and Animal Aquatic Health Unit of the Faculty of Veterinary Medicine, En Azmi and En Zainal for their help and technical support in the laboratory work.

Last but not least, I would like to thank wholeheartedly whomever were involved in this project to make it successful.

May God shower the above cited individuals with success and honour in their life.

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LIST OF ABBREVIATIONS

%	Percent
/	Or
<	Less than
>	More than
A. testudineus	*Anabas testudineus*
BET	Basal Epithelial Thickness
cm	Centimeter
g	Gram
H&E	Hematoxylin & Eosin
ID	Interlamellar Distance
L	Liter
mg	Milligram
NSPSF	North Selangor Peat Swamp Forest
PAGE	Proportion of the secondary lamellar Available for Gas Exchange
SLL	Secondary Lamellar Length
SLW	Secondary Lamellar Width

ABSTRAK

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

CIRIAN GROS DAN HISTOPATOLOGI *ANABAS TESTUDINEUS* DARI PAYA GAMBUT DAN TERUSAN PENYUAP DI HUTAN PAYA GAMBUT SELANGOR UTARA, MALAYSIA

Oleh

NURNAJWA ATIQAH BINTI MD SALLEHUDDIN

2020

Penyelia: Dr. Mazlina Mazlan

Penyelia Bersama: Dr Mohd Fuad Matori, Dr Awang Hazmi Awang Junaidi

Adaptasi spesies ikan yang menghuni keadaan air ekstrim telah menarik banyak minat untuk kajian dalam bidang ini. Namun, tiada laporan mengenai perubahan morfologi dan histopatologi *Anabas testudineus* yang mampu bertahan hidup di Hutan Paya Gambut Selangor Utara (NSPF). Oleh itu, kajian ini melibatkan 13 ekor ikan dewasa *Anabas testudineus* yang diperoleh dari paya gambut dan terusan penyuap bertujuan untuk membandingkan perubahan gros dan histopatologi insang, hati dan ginjal ikan ini dari kawasan paya gambut yang berasid (pH 3.24) dan terusan penyuap yang bersifat seperti persekitaran semulajadi (pH 6.42) hutan simpan. Hasil menunjukkan bahawa tiada perbezaan yang bererti ($p > 0.05$) dalam purata berat badan, jumlah panjang badan dan panjang insang, hati dan ginjal antara kedua-dua kumpulan. Analisis morfometrik insang ikan dari keadaan berasid menunjukkan panjang lamela sekunder yang lebih pendek (SLL), ketebalan epitel basal yang lebih rendah (BET), jarak antara lamela yang lebih pendek (ID) yang mengurangkan jumlah keseluruhan ruang mati, lebar lamela sekunder yang lebih tebal (SLW). Bahagian lamela sekunder yang tersedia untuk pertukaran gas (PAGE) lebih tinggi pada ikan dari paya gambut (42.6%) daripada kawasan terusan penyuap (34.3%). Secara gros, ikan yang ditangkap dari paya gambut juga menunjukkan warna kusam, legap, lebih gelap, dan epidermis yang lebih licin. Selain itu, ikan-ikan ini juga menunjukkan bentuk ganoid sisik ikan yang kurang jelas, hiperemik dan seperti lut sinar pada bahagian ventrikal perut, dan hati berwarna khas oren terang dengan margin bawah yang bulat dan kapsul berkilau. Penilaian histopatologi menunjukkan perbezaan yang signifikan ($p < 0.05$) dalam parameter seperti pemisahan epitelium dan hiperplasia sel goblet pada insang, pembengkakan hepatosit atau degenerasi hidropik dan pemvakuolan lipid intrasitoplasma di hati, epitelium tubular yang yang piknotik dan degenerasi hidropik tubul renal antara kedua-dua kumpulan. Kesimpulannya, ikan *Anabas* dari 2 keadaan yang berbeza menunjukkan perubahan hati secara kasar dan histopatologi pada insang, hati dan ginjal. Oleh itu, persekitaran paya gambut yang berasid mempunyai kesan dalam perubahan gros dan histopatologi organ terpilih *Anabas testudineus*.

Kata kunci: *Anabas testudineus*, air pH berasid, gros, histopatologi, bahagian lamela sekunder yang tersedia untuk pertukaran gas (PAGE).

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine UPM in partial fulfillment of the course VPD 4999 – Final Year Project.

GROSS AND HISTOPATHOLOGICAL CHARACTERISTICS OF *ANABAS TESTUDINEUS* ACQUIRED FROM PEAT SWAMP AND FEEDER CANAL OF PEAT SWAMP FOREST IN NORTH SELANGOR, MALAYSIA

by

NURNAJWA ATIQAH BINTI MD SALLEHUDDIN

2020

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Co-supervisors: Dr Mohd Fuad Matori, Dr Awang Hazmi Awang Junaidi

The adaptability of fish species inhabiting extreme water condition has intrigued many studies in this area. However, there is no report on the morphological and histopathological changes of *Anabas testudineus* surviving in the North Selangor Peat Swamp Forest (NSPF). Hence, this study involved 13 adult *Anabas testudineus* fish procured from peat swamp site and feeder canal with the aim to compare the gross and histopathological changes of gills, liver and kidneys of these fishes in acidic (pH 3.24) and natural environment (pH 6.42) part of the forest reserve. Results showed no significant differences ($p>0.05$) in mean body weight, total body length and mean length of gills, liver and kidney between the two groups. Gill morphometric analysis in fishes from the peat swamp revealed shorter secondary lamellar length (SLL), less basal epithelial thickness (BET), shorter interlamellar distance (ID) which reduces total size of dead space, thicker secondary lamellar width (SLW). The proportion of the secondary lamellar available for gas exchange (PAGE) is higher in fishes from the peat swamp (42.6%) than those from the feeder canal (34.3%). Grossly, fishes captured from the peat swamp also displayed dull, opaque, darker colouration, more slimy and slippery epidermal. Additionally, these fishes displayed less well-defined ganoid shaped of the scale, hyperaemic and translucent ventral abdomen and the liver were peculiar bright orange in colour with rounded inferior margin and glistening capsule. Histopathological assessment revealed significant differences ($p<0.05$) in parameters such as detachment of epithelium and hyperplasia of goblet cells in gills, hepatocyte swelling or hydropic degeneration and intracytoplasmic lipid vacuoles in liver, pyknotic tubular epithelium and hydropic degeneration of renal tubules in kidneys between the two groups. In conclusion, *Anabas* fishes from two different sites showed changes in the liver grossly and histopathologically in gills, liver and kidneys. Therefore, the peat swamp environment has an impact on the gross and histopathology of selected organs of *Anabas testudineus*.

Keywords: *Anabas testudineus*, acidic pH water, gross, histopathology, proportion of the secondary lamellar available for gas exchange (PAGE).

1.0 INTRODUCTION

1.1 Study Background

The freshwater fish, *Anabas testudineus*, is an economically important and nutritionally valuable food fish in Asian countries like Malaysia, Thailand, India, Philippines, and Bangladesh (Hossain *et al*., 2015). It can be found in swamps, stream, canals, paddy fields, small rivers and ponds which get flooded water or link to open waterways. *Anabas testudineus* can also survive in dry season by burying itself in the mud (Rahman, 1989) or by staying in pools associated with submerged shrubs and woods (Sokheng *et al*., 1999). With sufficient humidity, it is also able to travel several hundred meters of distance per trip on land (Davenport and Martin, 1990). This unique air breathing capacity and ability to survive on land is due to the fact that it has accessory breathing organs which can absorb oxygen in the air from above the water surface (Taylor *et al*., 2010). The labyrinth which is located in the upper part of its gill chambers enables the fish to take oxygen directly from the air (Hughe, Dube and Munshi, 1973).

Anabas testudineus has also been found to withstand habitat with extremely unfavourable water conditions (Pethiyagoda, 1991). It has been found inhabiting unused mine with a very acidic pH range of 2-4 (Saenphet *et al*., 2009) and brackish water ecosystem (Sarkar *et al*., 2005). However, fish physiology is dependent on internal and external factors. Internal factors synchronize its body functions such as nervous, endocrinological and neuroendocrinological while the two external factors that determine ecological factors include temperature, salinity, and photoperiod and limiting factors which are ammonia level, dissolved oxygen and pH value (Sharif *et al*., 2015). Extreme condition such as high acidity of water and low dissolved oxygen may result in lesion formation either grossly or histopathologically especially on the gill filaments. This is due to persistent and direct contact of fish gills to the environment and thus are directly affected by contaminants (Saenphet *et al*., 2009). Kidney tissues of *Anabas testudineus* acquired from polluted river showed necrosis of tubular epithelium, cloudy swelling of epithelium cells of renal tubules, narrowing of the tubular lumen, and contraction of the glomerulus and expansion of space inside Bowman's capsule compared to those taken from an unpolluted river (Joseph *et al*., 2012). It has also been found that gill specimens from control fish showed a typical structural organization of the lamella while those exposed to acid water had several histological alterations, namely desquamation of lamellar epithelium, fusion of the lamellae and lamellar aneurisms. (Saenphet *et al*., 2009). Hence, it can be concluded from the literature that *Anabas testudineus* exposed to extreme conditions directly and continuously develop gross and histopathological changes to the kidneys, liver and gills.

In Malaysia, a wide range of aquatic fished inhabits the North Selangor Peat Swamp forest. It is a flat coastal plain encompassing four forest reserve namely Raja Musa Forest Reserve, Sungai Karang Forest Reserve, Sungai Dusun Forest Reserve and portion of Bukit Belata Extension Forest Reserve. Due to economic development, the Peat Swamp Forest reserves areas has been encroached by agricultural activities dividing it into reserved peat swamp forest, oil palm plantations and paddy fields. The water quality in this forest reserve is rather unique in nature where the peat swamp has the lowest pH value compared to the other areas and the forest black water environment only permits a limited number of fish species to survive. However, *Anabas testudineus* is found in all areas of the forest reserve both in the extreme and its natural environment noting it as eurytopic, able to withstand acid to neutral or slightly alkaline water conditions (Integrated Management Plan for North Selangor Peat Swamp forest 2014-2023 Vol.1, 2014). Although numerous studies have been made on *Anabas testudineus* inhabiting acidic environment, none have focused on the morphological and histopathological changes of this species in the North Selangor Peat Swamp Forest. Hence, this study will focus on the morphological and histopathological changes of gills, liver and kidneys of *Anabas testudineus* inhabiting both the acidic and almost neutral part of the forest reserve.

1.2 Justification on Selection of Species

Aquatic fish inhabits the North Selangor Peat Swamp forest abundantly. However, *Anabas testudineus* is found in both extreme and its natural environment (Integrated Management Plan for North Selangor Peat Swamp forest Vol.1, 2014). This can be explained by its special adaptive mechanism from the aspect of physiological function and manners to maintain its homeostasis under unfavourable condition (Trattner *et al*., 2008). The adaptability of this species when exposed to extreme conditions will cause gross and histological changes and because of direct and continuous contact with this environment, fish kidney and gills are directly affected. Studies have shown that *Anabas testudineus* acquired from polluted river shows necrosis of tubular epithelium, cloudy swelling of epithelium cells of renal tubules, narrowing of the tubular lumen, and contraction of the glomerulus and expansion of space inside Bowman’s capsule were observed in kidney tissues compared to *A. testudineus* from an unpolluted river (Babu *et al*., 2012). Gills in the control *Anabas testudineus* fish showed a typical structural organization of the lamella while Anabas fish exposed to acid water had several histological alterations, namely desquamation of lamellar epithelium, fusion of the lamellae and lamellar aneurisms (Saenphet *et al*., 2009). Therefore, findings from previous studies have proven the adaptability of this unique fish species inhabiting extreme condition.

1.3 Objectives

1) To evaluate the gross lesions from gills, liver, and kidneys of *Anabas testudineus* acquired from extreme condition (peat swamp site) and its natural environment (feeder canal) in North Selangor Swamp forest.

2) To evaluate the histopathological lesions from gills, liver, and kidneys of *Anabas testudineus* acquired from extreme condition (peat swamp site) and its natural environment (feeder canal) in North Selangor Swamp forest.

1.4 Hypotheses

1.4.1 Gross Changes:

Ho_{11}: There is absence of gross changes in gills, liver, and kidneys of *Anabas testudineus* that lives under extreme condition (peat swamp site) compared to those from natural environment (feeder canal) in North Selangor Peat Swamp forest.

Ha_{11}: There is presence of gross changes in gills, liver, and kidneys of *Anabas testudineus* that lives under extreme condition (peat swamp site) compared to those from natural environment (feeder canal) in North Selangor Peat Swamp forest.

1.4.2 Histopathological Changes:

Ho_{21}: There is absence of histopathological changes in gills, liver and kidneys of *Anabas testudineus* that lives under extreme condition (peat swamp site) compared to those from natural environment (feeder canal) in North Selangor Peat Swamp forest.

Ha_{21}: There is presence of histopathological changes in gills, liver, and kidneys of *Anabas testudineus* that lives under extreme condition (peat swamp site) compared to those from natural environment (feeder canal) in North Selangor Peat Swamp forest.

2.0 LITERATURE REVIEW

Anabas testudineus (climbing perch) or locally known as 'Puyu' can tolerate extremely unfavourable water conditions associated mainly with turbid and stagnant waters (Pethiyagoda, 1991). This fish species is also able to populate in a highly acidic and toxic abandoned lignite mine (Saenphet *et al*., 2009). This finding conforms to the meaning of extremophiles defined as organisms that can survive in extreme environments which also acts as an indicator to the possible range of extremity that could support life. The extremity includes temperature, pH, salinity, hydrostatic pressure and ionizing radiation, as well as low oxygen tension, desiccation and the presence of heavy metals (Irwin, 2010). Hence, the ability of *Anabas testudineus* (climbing perch) to survive in extreme water conditions has drawn attention to the studies of samples taken from their gills, liver and kidneys to note for significant gross and histopathological changes. Histological alterations are more sensitive and occurred earlier in comparison to reproductive and developmental changes (Poleksic *et al*., 2010). On the other hand, histopathological biomarkers incorporate biotic factors and water quality that provides the whole view of fish status and makes it a reliable marker for environmental stress (Stentiford *et al*., 2013). *In situ* tests whereby healthy animals are taken to the field and exposed directly to the potentially contaminated environment, have frequently been used in environmental studies in fish (Camargo and Martinez, 2006). However, on site method is preferable in this study in order to obtain an accurate observation and recording of the gills, liver and kidneys samples in order to compare the differences if any between the two environments.

2.1 Gross and Histopathological Changes

2.1.1 Respiratory System: Gills

Fish gills is the first organ to which any pollutant comes into contact and highly sensitive to alteration in composition of the environment. Consequently, injury to gill epithelium is a common response observed in fish exposed to a variety of contaminants (Samuel *et al*., 2018). Based on findings of control group of *Anabas testudineus* fish in which no stresses, toxicants or pollutants are being introduced, the gill arches showed normal arrangement pattern of primary lamellae and secondary lamellae that projects from the lateral sides of primary lamellae (Samuel *et al*., 2018). Layer of simple squamous epithelium which is the active exchange pillar cells, covered the surface of the secondary lamellae. The primary lamellae contain a rigid mass of cartilaginous tissues in its core with traces of vascular channels (Olurin *et al*., 2016). One of the alterations of the gills include elevating mucous production due to the effects of acidic water pH exposure (Balm and Pottinger, 1993). The effects of mucous accumulation may cause suffocation and other alteration like breakdown of gills structure done from a study on zebrafish (*Danio rerio*) exposed to extreme acidic water (pH 2.0–3.5) (Packer and Dunson, 1972). On the other hand, the most common gill changes observed in climbing perch acquired from acidic environment were desquamation in secondary lamellae, lifting of the lamellar epithelium and telangiectasia. However, there was no gill abnormalities observed in the gills of the control farmed fish (Saenphet *et al*., 2009). Those findings can also be supported by an experimental study, which demonstrated epithelium lifting and oedema on the fish gills seen in all treatment groups of pH water parameters except for pH 7, which demonstrated no histopathological changes (Salleh *et al*., 2017)

2.1.2 Hepatobiliary System: Liver

Fish liver is considered to be a target organ for many biological and environmental parameters that can alter liver structure and metabolism which might be due to food, pollutants, toxins, parasites, and microorganisms. In an experimental study, *Anabas testudineus* developed severe pathological changes such as dispersed blood cells and vessels, damage in acinar cells of hepatopancreas, detachment of hepatopancreatic acinar cells from hepatocytes after almix intoxication in a laboratory condition (Samanta *et al*., 2015). *Anabas* fish exposed to sewage water showed marked degenerative and necrotic changes (Narain, Srivastava, and Singh, 1990). Physiologically, histology of liver from *Anabas testudineus* exhibited typical parenchymatous appearance whereby each tube was surrounded by a very thin connective tissue capsule extended as a trabecular into the body of the lobes and divided into irregularly shaped lobules. The hepatocytes are polygonal shaped with central spherical nucleus and arranged as irregular cord-like structure in the section separated by sinusoids (Samuel *et al*., 2018). However, due to acidic pH, there was alteration of the liver parenchyma, such as vacuolization and necrosis. These alterations are often associated with a degenerative-necrotic condition (Myers *et al*, 1987). Finding from previous study also presented diffuse changes in the hepatic parenchyma with oedematous condition, vacuolization, marked degeneration and constriction of the sinusoids in the liver of *Anabas testudineus* due to the effect of toxicant pollutants. Haemorrhages leading to extensive necrosis of hepatic cells were also noticed (Samuel *et al*., 2018).

2.1.3 Renal System: Kidney

Kidneys of fish received the largest proportion of post-branchial blood and thus, renal lesions may be good indicators of environmental pollution (Ortiz *et al*., 2003). Lesions in kidneys of *A. testudineus* exposed to acidic water had similar alterations as in previous studies (Saenphet *et al*., 2009). Kidneys showed various degenerative and necrotic changes under exposure to sewage or polluted water (Narain and Srivastava, 1990). Histopathological changes of kidneys in a contaminated environment revealed presence of hypertrophy of renal cells, reduced inter-tubular spaces, invariably dilated tubular lumen, formation of vacuoles, necrosis and degeneration of renal components. Moreover, hyperplasia was seen in the tubular epithelium with necrotic changes characterized by karyorrhexis and karyolysis of the nuclei of the affected cells. Parenchymatous cells revealed marked oedema and congestion of sinusoids with interstitium markedly infiltrated with mononuclear cell as compared to the control fish (Samuel *et al.*, 2018). *Anabas testudineus* inhabiting polluted river also showed necrosis of tubular epithelium, cloudy swelling of epithelium cells of renal tubules, narrowing of the tubular lumen, and contraction of the glomerulus and expansion of space inside Bowman's capsule in kidney tissues compared to those taken from unpolluted river (Babu *et al*., 2012).

3.0 MATERIALS AND METHOD

3.1 Study Area

This study was conducted in North Selangor Peat Swamp Forest (NSPSF), a flat coastal plain in humid tropical zone with heavy rainfall, high humidity and temperature. NSPSF has a total area of 81,304 hectares, which consists of four forests reserve including Raja Musa Forest Reserve, Sungai Karang Forest Reserves and partially Bukit Belata Extension Forest Reserve, and Sungai Dusun Forest Reserve/Sungai Dusun Wildlife Reserve. These forests reserves are formed between Bernam river to the north, Selangor river to the south, and Tengi river cutting across it.

3.1.1 Peat Swamp Site

Previously, there was an estimated of 500 km of ex-logging canals. The specified area studied for peat swamp site with blackwater water condition is at Jalan Sg. Panjang, which is one of the extensive networks of canals located near to the eastern part of Sungai Karang Forest Reserve. This water condition occurs in ditches that run almost parallel along both sides of the trunk road with moderately fast to slow streams. Water depth range from 45 to 200 cm at the deepest parts and the water level rises and falls in tandem with rainfall. The substrate is peat, submerged logs, and in some parts gravel or sand.

3.1.2 Feeder Canal

Feeder canal carries Bernam river, the main river flowing in Northern Selangor to the main source of water for Tanjong Karang Irrigation Project towards the rice growing areas located along the southwestern part of NSPSF. A large irrigation canal near the Tanjong Karang Irrigation project connects Tengi river, that merges with blackwater from NPSF to Sg Bernam.

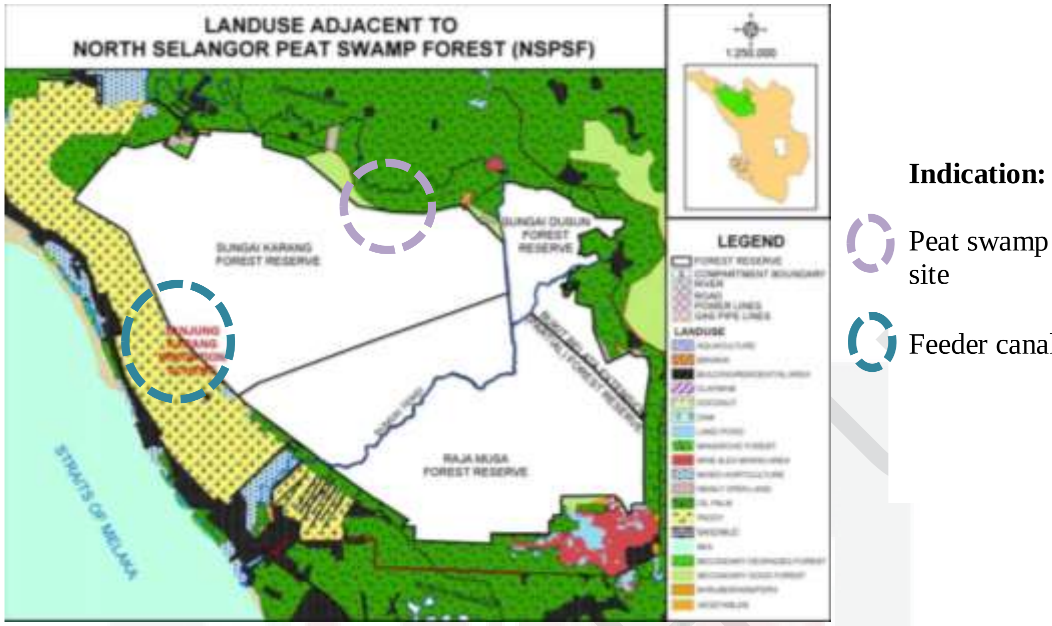


Figure 1: The Geographical Mapping of Peat Swamp Forest (Parish *et al*., 2014)

3.2 Water Quality

Water parameters were measured using Thermo Scientific Orion Star A325 pH/Conductivity Portable Multiparameter Meter and YSI 550A Dissolved Oxygen Meter at three different sites in order to obtain the means of pH, dissolved oxygen (DO) (mg/L) and temperature (°C) at two different locations which included the peat swamp site and feeder canal.

3.3 Sample Collection and Morphological Evaluation

Six adult *Anabas testudineus* fish acquired from peat swamp site and seven adult *Anabas testudineus* fish acquired from feeder canal were randomly captured using casting nets and fishing rod. The fishes were physically restrained, body weight and total body length were measured using weighing scale and measuring ruler respectively followed by morphological evaluation. Pithing technique was done as a humane method of killing fish by inserting scalpel blade at the craniodorsal area until it reached the spinal cord. The sex of each fish were noted and samples of gills, liver and kidneys were obtained from all 13 fishes on-site.

3.4 Gross Examination and Histopathological Slides Preparation

The gross lesions were examined and recorded on samples of gills, liver and kidneys before fixed in 20% buffered formalin for 4 hours continued by 10% buffered formalin for 24 to 48 hours. All samples were transferred to 70% alcohol until the processing time except for the gills which were treated with formic acid for 6 hours before the alcohol pocedure. Gills, liver, and kidneys from 13 fishes were cut using scalpel blade and kept in labeled casettes which were stored in 70% alcohol. All the samples were dehydrated, diaphenizated and impregnated during tissue processing using the Shandon Citadel 1000® automatic tissue processor for approximately 16 hours before embedding process. During embedding with paraffin, the organs were arranged into the stainless steel base mould with the aid of heated twizzers before the paraffin solidification. Prior to tissue sectioning, paraffin blocks were freezed for at least 24 hours. Sectioning was done using rotary microtome at 5 µm thickness until the paraffin ribbons which contained the respective samples were formed and transferred to water bath at 45°C. Stretched ribbon was scooped up onto a clean slide, labelled and kept dry on slide drying hotplate. Dried labelled slides were stained using Harris' Hematoxylin and Eosin (H&E) staining method which begins by submerging slides in xylene followed by 100% alcohol, and 70% alcohol where all agents were timed for 5 minutes. Slides were rinsed under running water in 10 dips before being submerged for 5 minutes in Hematoxylin and rinsed again with water 3 to 5 times. Slides were then dipped in 1% acid alcohol for only 3 seconds followed by rinsing with running tap water for 5 minutes and submerged in Eosin for 1 minute. Decolourization of slides using sprayed 95% alcohol was done before slides were rinsed under running tap water 5 to 10 seconds. Second last step was to spray the slides again with 95% alcohol, cleaned and dried before being mounted with DPX. The slides of gills, liver and kidneys were viewed under light microscope at total magnification of 40X, 100X, 200X, and 400X.

3.5 Histopathological Description and Evaluation

Slides of the entire gill arch, liver tissues (1.6mm^2), and kidneys (5mm^2) of each *Anabas testudineus* were observed under different magnifications power (40X, 100X, 200X and 400X) under the light microscope. Histopathological alterations in gills, liver and kidneys were evaluated using the protocol described by Mauricio *et al*. (2018) and Rey *et al*. (2020), which is a modification to Bernet *et al*. (1999).

Evaluation on gills alteration was done based on protocol by Mauricio *et al*. (2018) which consists of:

1. Lamellar oedema: abnormal accumulation of fluid in tissue space of lamellar.
2. Hyperplasia of secondary lamellae: enlargement of secondary lamellae by a greater number of cells without change in volume of the cells.
3. Congestion of secondary lamellae: blockage of blood leaving the secondary lamellae leading to accumulation of erythrocyte in venous circulation.
4. Epithelium architectural and structural alteration: changes in tissue structure, shape and arrangement of gill epithelium.
5. Fusion of secondary lamellae: may be partial or complete fusion of secondary lamellae due to hyperplasia or hypertrophy.

6. Detachment of epithelium: epithelium appears detached from the underlying cores of blood spaces and pillar cell of secondary lamellar.

7. Hyperplasia of goblet cells: increase in number of goblet cells in secondary lamellae

8. Telengiectasia: reversible swollen blood vessels in the secondary gill lamellae caused by rupture of the pillar cells or its union in gills

Assessment on liver alterations were done based on protocol by Rey *et al*. (2020) which consists of:

1. Congestion of hepatopancreas: accumulation of erythrocyte in a dilated capillaries and veins of hepatopancreas.

2. Hepatocyte swelling or hydropic degeneration: cell swelling refers to crowded and disarranged cell which appears rounded while hydropic degeneration is cell which is severely swollen with formation of small and clear vacuoles in cytoplasm.

3. Pyknotic hepatocytes: nucleus appears slightly smaller, and homogeneously very dark basophilic of hepatocytes.

4. Activation of melanomacrophage centre: presence of aggregates of highly pigmented phagocytes in liver.

5. Intracytoplasmic lipid vacuoles: abnormal accumulation of fat vacuoles in cytoplasm of hepatocytes.

Evaluation on kidneys alterations were done based on protocol by Rey *et al*. (2020) which consists of:

1. Congested interstitium: accumulation of erythrocyte in a dilated capillaries and veins of renal interstitium

2. Pyknotic tubular epithelium: nucleus appears slightly smaller, and homogeneously very dark basophilic of renal epithelium

3. Hydropic degeneration renal tubules: severely swollen cell with formation of small and clear vacuoles in cytoplasm of renal epithelium

4. Hyaline droplet degeneration and granular/hyaline casts in the tubular lumina: small, eosinophilic structures in cytoplasm of renal tubules with presence of casts in lumen

5. Activation of melanomacrophage centres: presence of aggregates of highly pigmented phagocytes in renal

Tissue samples from liver and kidneys were divided into three equal areas (0.5×3.2 mm) and (0.3×1.67 mm) respectively to avoid overlapping areas when examined at magnifications of 200X and 400X. The three equal areas were examined and recorded in order to take the average scoring lesion in each alteration observed. For gills, the number of gill filaments with a particular alteration (examined under 200X and 400X magnifications) were divided by the total number of gill filaments (examined under 10X magnification).

The percentage of the affected gill filaments, liver and kidneys containing a particular alteration were determined by the score value ranging from 0 to 6 depending on severity: (0) unchanged 0%; (2) mild occurrence 21-30%; (4) moderate occurrence 41-50%; and (6) severe occurrence (diffuse lesion) 60% or more. Intermediate values of the scoring were also considered.

3.6 Gill Morphometric Analysis

Each gill filaments of *Anabas testudineus* acquired from peat swamp site and feeder canal were measured based on the morphometric analysis done by Nero *et al*. (2006). The aspects of measurements consist of secondary lamellar length (SLL) and width (SLW), interlamellar distance (ID), and basal epithelial thickness (BET) whereby it represents the major dimensions of gill tissue influencing the diffusion distance (gas exchange) in fish (Hughes and Perry, 1976). For SLL, two measurements were made on every sixth secondary lamellae and its adjacent on each filament appeared on photomicrograph while for BET, three measurements were made on each filament appeared on photomicrograph; one measurement on top, bottom and centre of each filament to get the average. For ID, three measurements were made on every third to fourth secondary lamella and its adjacent appearing on each filament; the lamellar tip, the centre of the lamellae, and the base of the lamellae. For SLW, three measurements were made on every fourth secondary lamella and its adjacent appearing on each filament; the lamellar tip, the centre of the lamellae, and the base of the lamellae. The proportion of the secondary lamellae available for gas exchange (PAGE) was averaged for each filament of fish and calculated using the equation PAGE (%) = 100 × (mean SLL/ (mean BET+ mean SLL)). All gill morphometric indices are presented as mean ± standard deviation. Photomicrographs of gill tissue were taken using a digital camera (Nikon 9000) under high power magnification (200X and/or 400X) in a Nikon TE3000 light microscope.

3.7 Statistical Analysis

Independent sample T-test was done to determine whether there is statistical evidence that the means body weight, total body length, mean length of gills, liver and kidneys of *Anabas testudineus* between acidic and natural environment pH conditions are significantly different. Mann Whitney U Test was done to determine statistically significant difference in the histopathological lesions in gills between the two groups of *Anabas* fish taken from different pH conditions.

4.0 RESULTS

4.1 Water Chemistry

Comparison of the mean values of selected water quality parameters which include temperature, pH and dissolved oxygen are shown in Table 1.

Table 1: pH, Dissolved Oxygen (DO), and Temperature (°C) of peat swamp site and feeder canal (mean ± standard deviation)

Location	pH	DO (%)	DO (mg/L)	Temperature (°C)
Peat Swamp site	3.24 ± 0.01^a	58.7 ± 3.21^a	5.06 ± 0.10^a	27.4 ± 0.20^a
Feeder canal	6.42 ± 0.31^b	68.0 ± 2.00^b	5.62 ± 0.17^b	31.0 ± 2.95^a

Mean values for each column with the same superscript do not differ significantly, p>0.05 (from independent sample T-test)

Significant difference was observed ($p<0.05$) between the mean values of pH and Dissolved Oxygen (DO) between peat swamp site and feeder canal. Peat swamp site has a significantly low dissolved oxygen (5.06 mg/L) and temperature (27.4°C) compared to the feeder canal with higher dissolved oxygen (5.62 mg/L) and temperature (31.0°C).

The peat swamp site has a lower pH (3.24) compared to feeder canal being the natural environment with almost neutral pH (6.42). This result is similar to findings in a previous research project that showed pH (3.72 ± 0.08) in peat swamp site while feeder canal pH (6.06 ± 0.26) (Integrated Management Plan for North Selangor Peat Swamp forest, 2014).

4.2 Gross Analysis

4.2.1 Body Weight and Total Body Length

Majority of the fish samples captured were male which covers up to 80% while female only consist of less than 20% (refer to Table 2 in Appendix A). From Table 3, *Anabas testudineus* fish acquired from the peat swamp has mean body weight of 15.00 g which is slightly higher than mean body weight of *Anabas testudineus* fish acquired from the feeder canal (10.86 g). Mean total body length of *Anabas* fish from the peat swamp (9.33cm) cm was slightly lower compared to the mean of total body length of *Anabas* fish from the feeder canal (9.47 cm).

Table 2: Body Weight (g) and Total Body Length (cm) of *Anabas testudineus* from Peat Swamp and Feeder Canal (mean ± standard deviation)

Fish Group	Body Weight (g)	Total Body Length (cm)
Peat Swamp	15.00 ± 6.32^{a}	9.33 ± 1.37^{a}
Feeder Canal	10.86 ± 4.06^{a}	9.47 ± 3.10^{a}

Mean values for each column with the same superscript do not differ significantly, p>0.05 (from independent sample T-test)

Based on Table 3, mean body weight and total body length between the two groups are not significantly different with p-value 0.181 and 0.922 respectively. Thus, the mean body weight and mean of total body length between the two groups of *Anabas* fish under acidic pH (peat swamp site) and almost neutral pH (feeder canal) are not statistically significant.

4.2.2 Gills, Liver and Kidneys Lengths

The length of each samples of gills, liver and kidneys were measured and compared between the two pH conditions. Mean and standard deviation for each group is presented in Table 4.

Table 3: Gill, Liver and Kidneys Length (cm) of *Anabas testudineus* between the Peat Swamp and Feeder Canal Groups (Mean ± Standard Deviation)

Fish Group	Gill length (cm)	Liver length (cm)	Kidney length (cm)
Acidic pH	1.28 ± 0.13^{a}	1.02 ± 0.08^{a}	1.32 ± 0.39^{a}
Almost Neutral pH	1.09 ± 0.32^{a}	0.79 ± 0.35^{a}	0.99 ± 0.27^{a}

Mean values for each column with the same superscript do not differ significantly, p>0.05 (from independent sample T-test)

Mean length of gills, liver and kidneys in *Anabas* fish from the peat swamp is higher compared to mean length of gills, liver and kidneys in *Anabas* fish from the feeder canal (refer to Table 4). However, the independent sample T-test results showed that the mean length of gills, liver and kidneys are not significantly difference with p-value of more than 0.05 between *Anabas* fish in different pH conditions.

4.2.3 Morphological Assessment

The *Anabas* fish from acquired from from peat swamp site has dull, opaque and darker colouration, more slimy and slippery epidermal, less well-defined ganoid shaped of the scale and the ventral part of its body appeared hyperaemic and ‘translucent-like’. However, these findings are not found in *Anabas testudineus* acquired from feeder canal (Figure 2).

Figure 2: Photograph of gross appearance in *Anabas testudineus* acquired from peat swamp site (a) has dull, opaque and darker colouration, more slimy and slippery epidermal, less well-defined ganoid shaped of the scale and the ventral part of its body appeared hyperaemic and 'translucent-like'. However, these findings were not found in *Anabas testudineus* acquired from the feeder canal (b).

4.2.4 Gross Findings of Gill, Liver and Kidneys

Figure 3 shows the gross findings of gill, liver and kidneys between the *Anabas* fish from the peat swamp and feeder canal. No gross changes between the gills and kidneys between *Anabas testudineus* acquired from feeder canal and peat swamp sites.

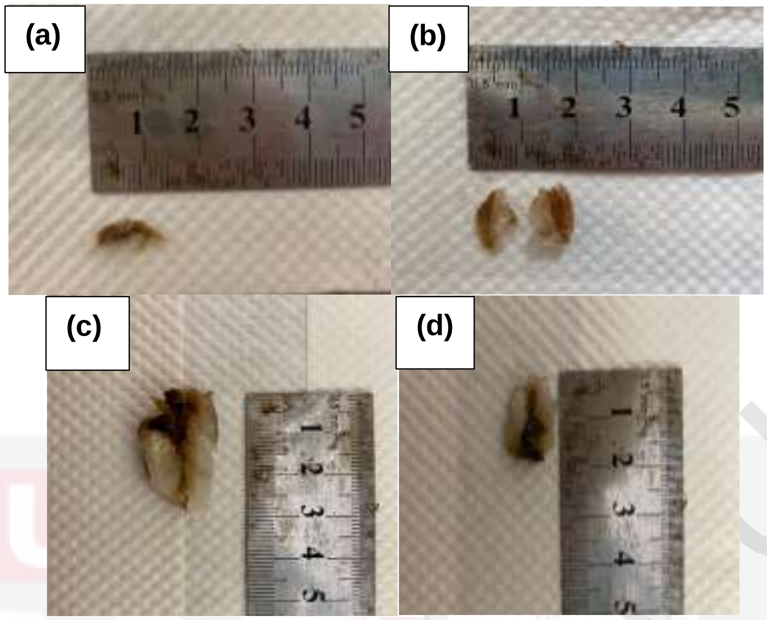


Figure 3: No gross changes between the gills and kidneys between *Anabas testudineus* acquired from feeder canal (b) and (d) and peat swamp site (a) and (c) respectively.

The hepatic parenchyma of *Anabas testudineus* acquired from the peat swamp appeared peculiarly bright orange colour with pale yellow on its posterior part. There was also presence of rounded inferior margin and glistening capsule. However, these findings are not found in liver of *Anabas testudineus* acquired from the feeder canal (Figure 4).

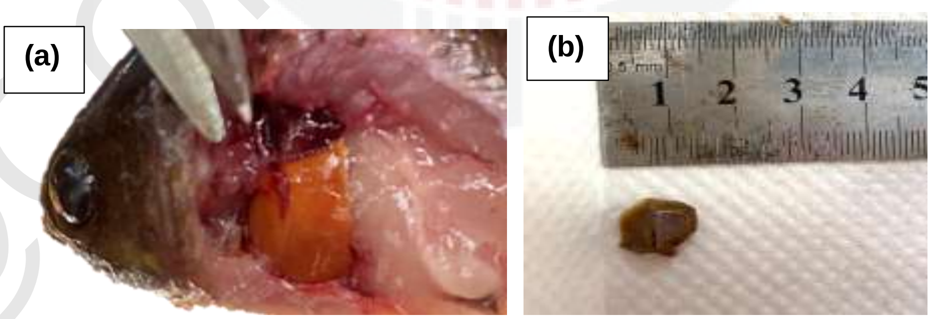


Figure 3: Hepatic parenchyma of *Anabas testudineus* acquired from the peat swamp (a) appeared peculiarly bright orange colour with pale yellow on its posterior part. There was also presence of rounded inferior margin and glistening capsule. However, these findings were not found in liver of *Anabas testudineus* acquired from feeder canal (b).

4.3 Histopathological Findings

4.3.1 Histopathology of Gill

There are several alterations found in comparison to *Anabas testudineus* captured from acidic peat swamp and less-acidic feeder canal group which include detachment of epithelium lining, telangiectasia, proliferation of goblet cells, hyperplasia and fusion of secondary lamellar with different scoring lesion as in Figure 7.

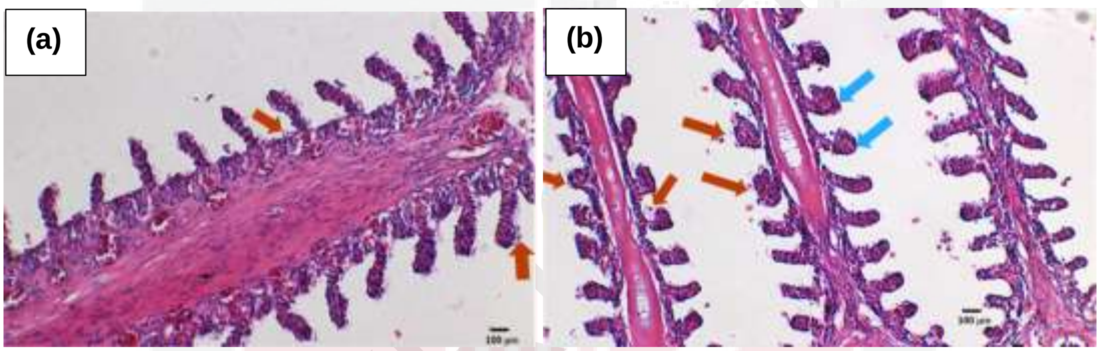


Figure 4: Photomicrographs of gills showing (a) short and stumpy secondary lamellar with detachment of epithelial lining (red arrow) in feeder canal fish (b) Presence of telangiectasia (blue arrow) and detachment of epithelial lining of secondary lamellar (red arrow) in peat swamp site fish.

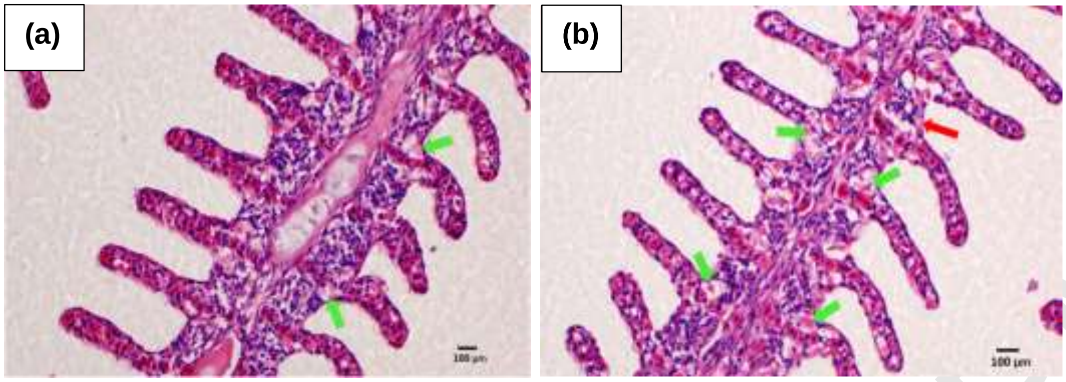


Figure 5: Photomicrograph of the gills in *Anabas testudineus* from feeder canal revealed (a) proliferation of goblet cells (green arrow) along the lengths of lamellae in a basal to apical direction while in peat swamp fish showed (b) proliferation of goblet cells (green arrow) and presence of chloride cells (red arrow) were observed.

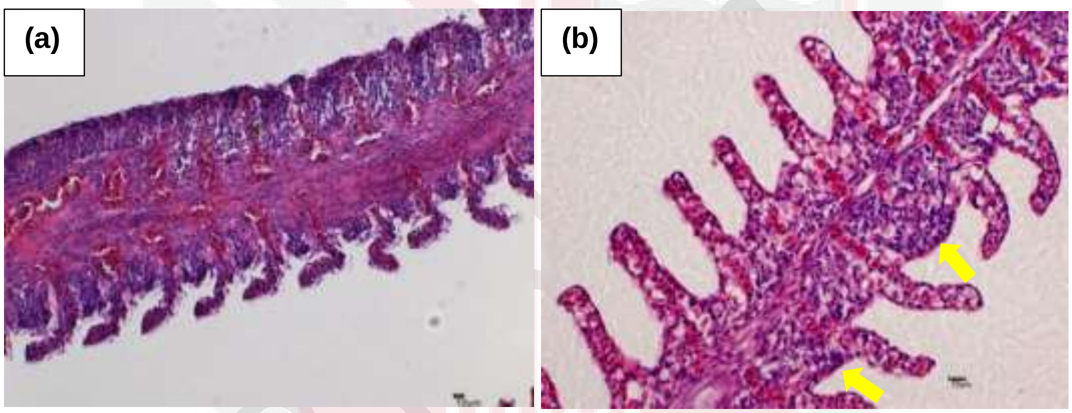


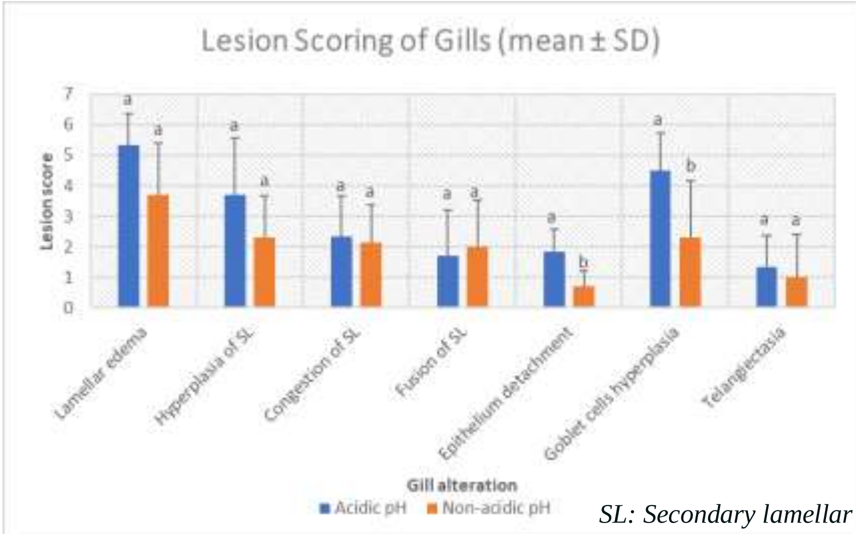
Figure 6: Photomicrograph shows hyperplasia and fusion of secondary lamellae with congested secondary lamellar in *Anabas testudineus* from feeder canal fish (a) while in *Anabas testudineus* from peat swamp fish (b) shows partial hyperplasia of secondary lamellar (yellow arrow).

4.3.2 Statistical Analysis of Histopathological Lesion in Gills

Gill alterations found in *Anabas* fish in the feeder canal group include presence of mild to moderate lamellar oedema with less than 40% of areas affected and mild occurrence of hyperplasia of secondary lamellae with around 20% of areas affected. Less than 30% affected gills have congested secondary lamellar, detachment of epithelium, fusion of secondary lamellae and hyperplasia of goblet cells. Meanwhile, telangiectasia is found to be less than 10% of areas of gills affected.

Nevertheless, *Anabas* fish from the acidic peat swamp group shows moderate occurrence of lamellar oedema and hyperplasia of goblet cells with around 50% of the gills affected. The alterations based on hyperplasia of secondary lamellae is about 40% from the area of gills affected. Most common finding is the presence of pavement cells that can be found having cuboidal shaped, large and polygonal nucleus lining the surface of secondary lamellae. Other alterations include congestion of secondary lamellae, fusion of secondary lamellae, detachment of epithelium, and telangiectasia affecting less than 20% of the gills of *Anabas* fish in acidic peat swamp pH.

Mann Whitney U test revealed that there is significant difference ($p<0.05$) on both alterations for detachment of epithelium and hyperplasia of goblet cells between two groups of fish in different pH condition.



Data with different letter (a or b) between groups differ significantly (P < 0.05) (from Mann Whitney U Test)

Figure 7: Lesion scoring of gills in *Anabas testudineus* for each alteration made based on protocol by Mauricio et al. (2018)

4.3.3 Histopathology of Liver

Several liver histopathological lesions were observed from the peat swamp and feeder canal group which includes intracytoplasmic lipid vacuolation, hepatocyte swelling, pyknotic hepatocytes and activation of melanomacrophage centre as shown in Figures 8-10. However, the scoring is differed between the two groups (refer to Figure 10).

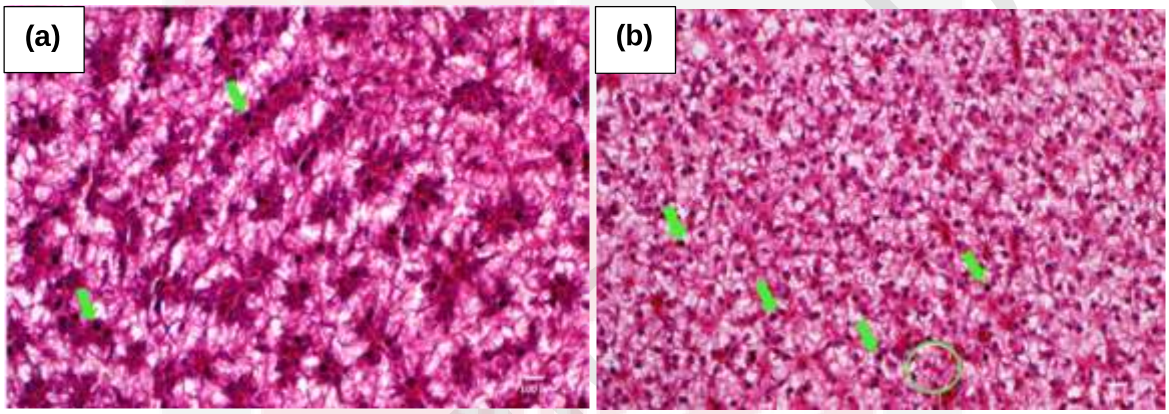


Figure 7: Photomicrograph of liver of *Anabas testudineus* from feeder canal group showing (a) nucleus of hepatocytes (green arrow) arranged collectively along the sinusoids due to presence of intracytoplasmic lipid vacuolation. Meanwhile, the liver of fish from peat swamp group showing (b) generalized intracytoplasmic lipid vacuoles, presence of hepatocyte swelling (green circle), and homogeneously very dark basophilic nucleus of pyknotic hepatocytes with dense chromatin (green arrow).

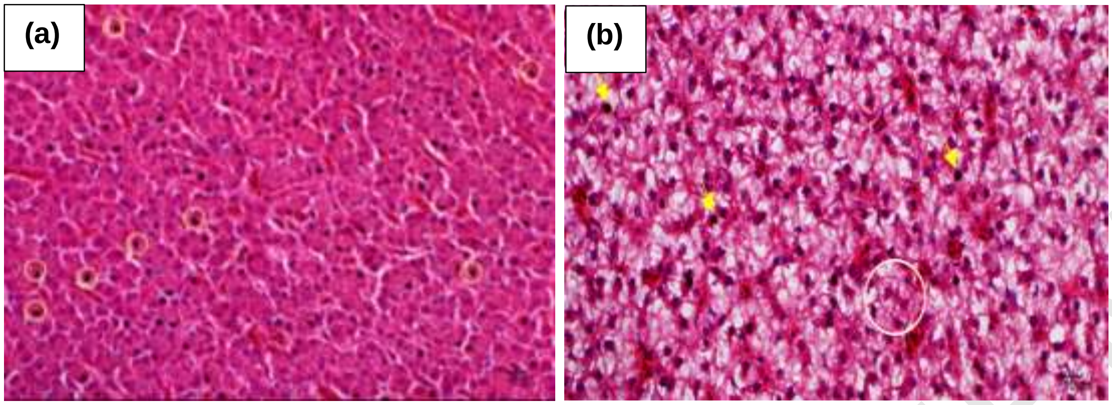


Figure 8: Photomicrograph of liver of *Anabas testudineus* from feeder canal group revealing (a) shrunken, eosinophilic cytoplasm, homogeneously very dark basophilic nuclei of pyknotic hepatocytes with dense chromatin (yellow circle). Meanwhile, for peat swamp group showing (b) generalized intracytoplasmic lipid vacuoles, presence of hepatocyte swelling (circle), and homogeneously very dark basophilic nuclei of pyknotic hepatocytes with dense chromatin (yellow arrow).

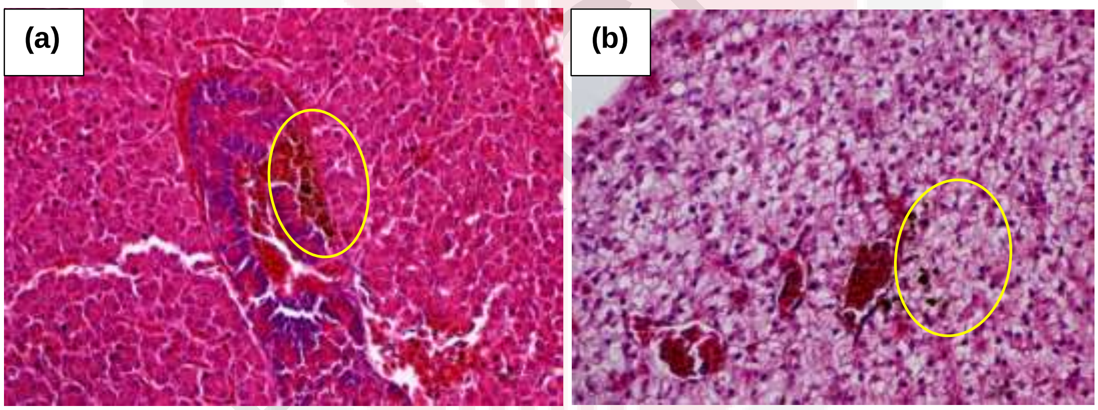


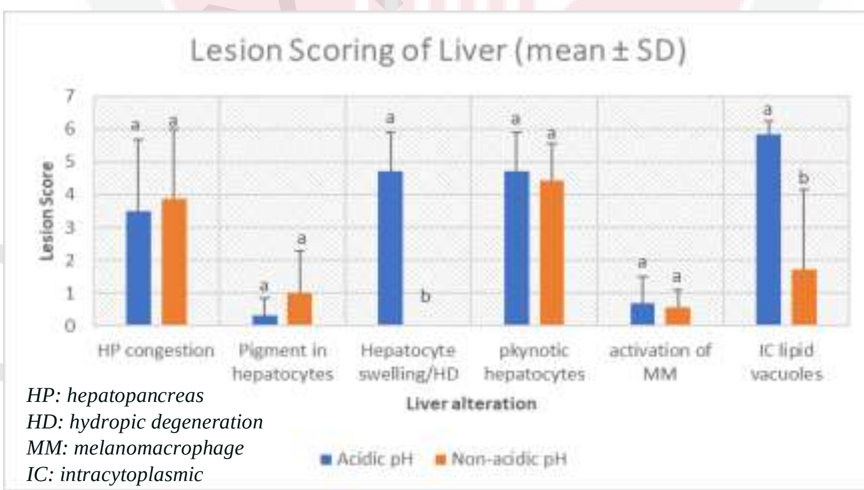
Figure 9: Photomicrographs of liver of *Anabas testudineus* from feeder canal group (a) and peat swamp group (b) showing highly pigmented aggregates of phagocytes namely melanomacrophage centre (yellow circle).

4.3.4 Statistical Analysis of Histopathological Lesion in Liver

In the *Anabas* fish from the feeder canal group, the histopathological lesions observed were presence of mild to moderate congestion of hepatopancreas and pyknotic hepatocytes with around 40% of areas affected. Meanwhile, there is very mild presence of pigmentations in the cytoplasm of hepatocyte, activation of melanomacrophage centre and intracytoplasmic lipid vacuoles which only affect less than 20% of areas of the liver from *Anabas* fish in feeder canal group. However, there is no change or none of the liver have hepatocyte swelling or hydropic degeneration.

For the peat swamp group, there is presence of moderately to severe intracytoplasmic lipid vacuoles with around 60% of areas of liver affected. This followed by presence of moderately occurrence hepatocyte swelling/hydropic degeneration and pkynotic hepatocytes. Congestion of hepatopancreas can also be seen mildly with less than 40% of areas of liver are affected. Meanwhile, there is a very mild activation of melanomacrophage and presence of intracytoplasmic pigments of hepatocytes from *Anabas* fish in peat swamp group.

Mann Whitney U test revealed that there are significant differences ($p<0.05$) on alterations for hepatocyte swelling/hydropic degeneration and intracytoplasmic lipid vacuoles between two groups of *Anabas* fish in different pH conditions.



Data with different letter (a or b) between groups differ significantly (P < 0.05) (from Mann Whitney U Test).

Figure 10: Lesion scoring of liver in *Anabas testudineus* for each alteration made based on protocol by Rey *et al*. (2020)

4.3.5 Histopathology of Kidneys

Kidneys in both peat swamp and feeder canal groups showed several histopathological changes which include presence of hyaline droplet degeneration and granular/hyaline casts in the tubular lumina, pyknotic tubular epithelium and hydropic degeneration with different scoring as in Figure 14.

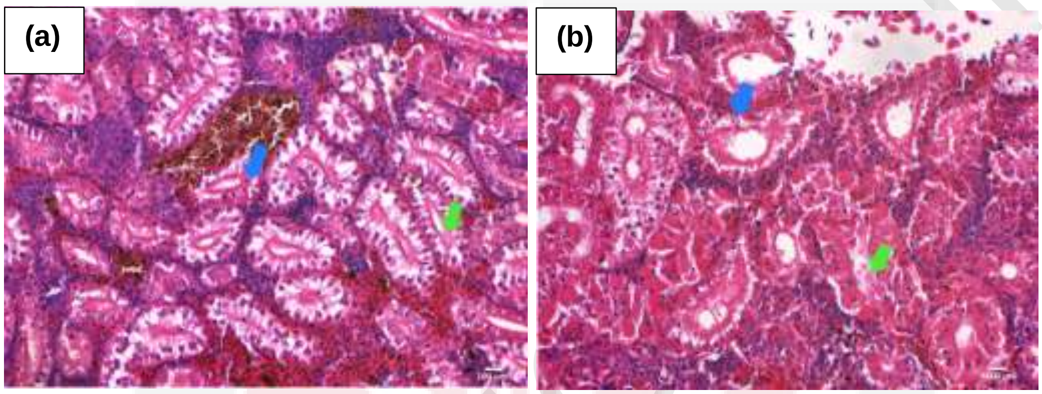


Figure 11: Photomicrograph of kidneys of *Anabas testudineus* in feeder canal group (a) and peat swamp group (b). Both groups showing presence of hyaline droplet degeneration and granular/hyaline casts in the tubular lumina (green arrow), and pyknotic tubular epithelium (blue arrow).

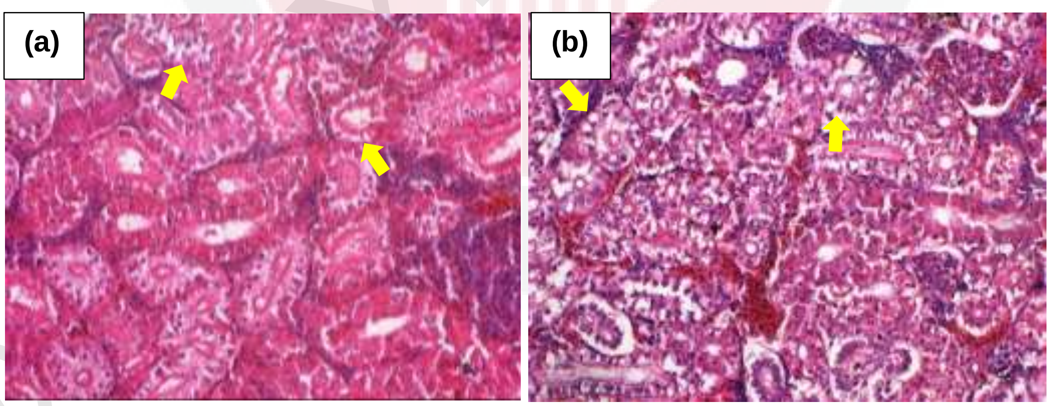


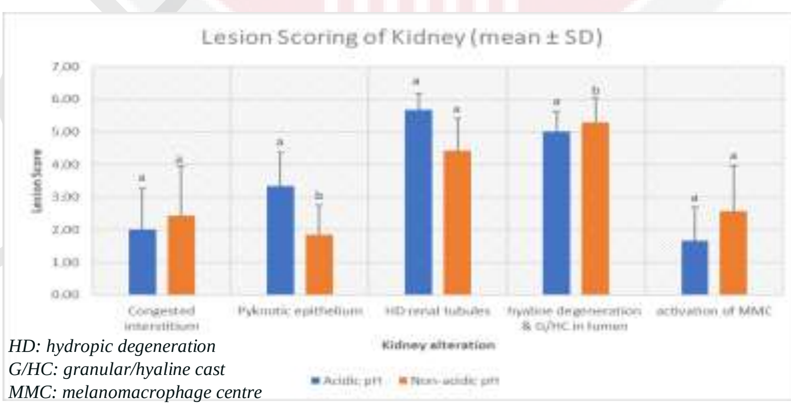
Figure 12: Photomicrograph of kidneys of *Anabas testudineus* in peat swamp group (a) and feeder canal group (b) showing hydropic degeneration of renal tubules (yellow arrow).

4.3.6 Statistical Analysis of Histopathological Lesion in Kidneys

For the *Anabas* fish from feeder canal group, moderate hydropic degeneration of renal tubules were noted with around 50% of the kidney areas affected. This is followed by the presence of hyaline droplet degeneration and granular/hyaline casts in the tubular lumina with around less than 50% areas affected. The remaining alterations only cover less than 20% of the areas affected in the kidney of *Anabas* fish in feeder canal group.

For the peat swamp group, severe hydropic degeneration of renal tubules were present with around 60% of the areas affected in the kidney. This is followed by the presence of hyaline droplet degeneration and granular/hyaline casts in the tubular lumina with 50% areas affected. The rest alterations only cover less than 30% of the areas affected from *Anabas* fish in peat swamp group.

Mann Whitney U Test results showed that there is significant difference between pyknotic tubular epithelium and hydropic of renal tubules with $p<0.05$ between *Anabas* fish in peat swamp and feeder canal groups.



Data with different letter (a or b) between groups differ significantly ($P < 0.05$) (from Mann Whitney U Test).

Figure 13: Lesion scoring of kidneys in *Anabas testudineus* for each alteration made based on protocol by Rey et al. (2020)

4.4 Gill Morphometric Analysis

Gill Morphometric Analysis was done based on protocol by Nero *et al*. (2006) which measures the Secondary Lamellar Length, Basal Epithelium Thickness, Interlamellar Distance, and Secondary Lamellar Width as shown in Figure 15.

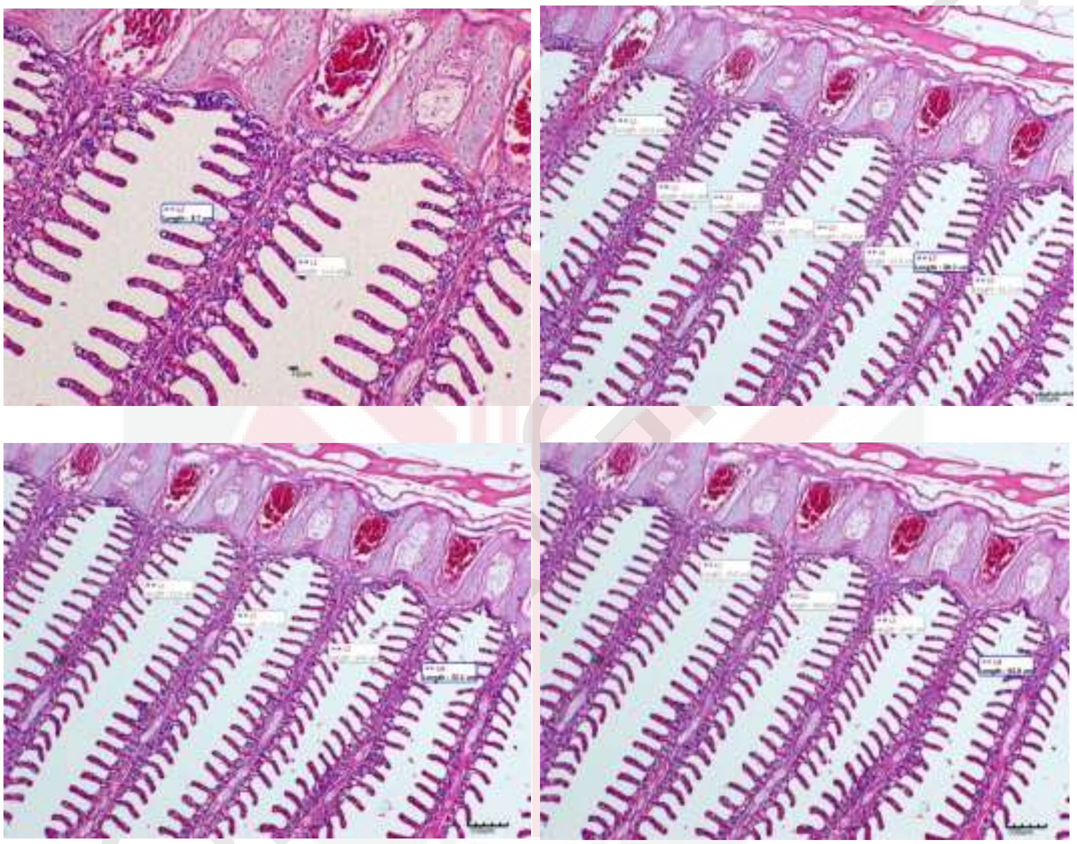
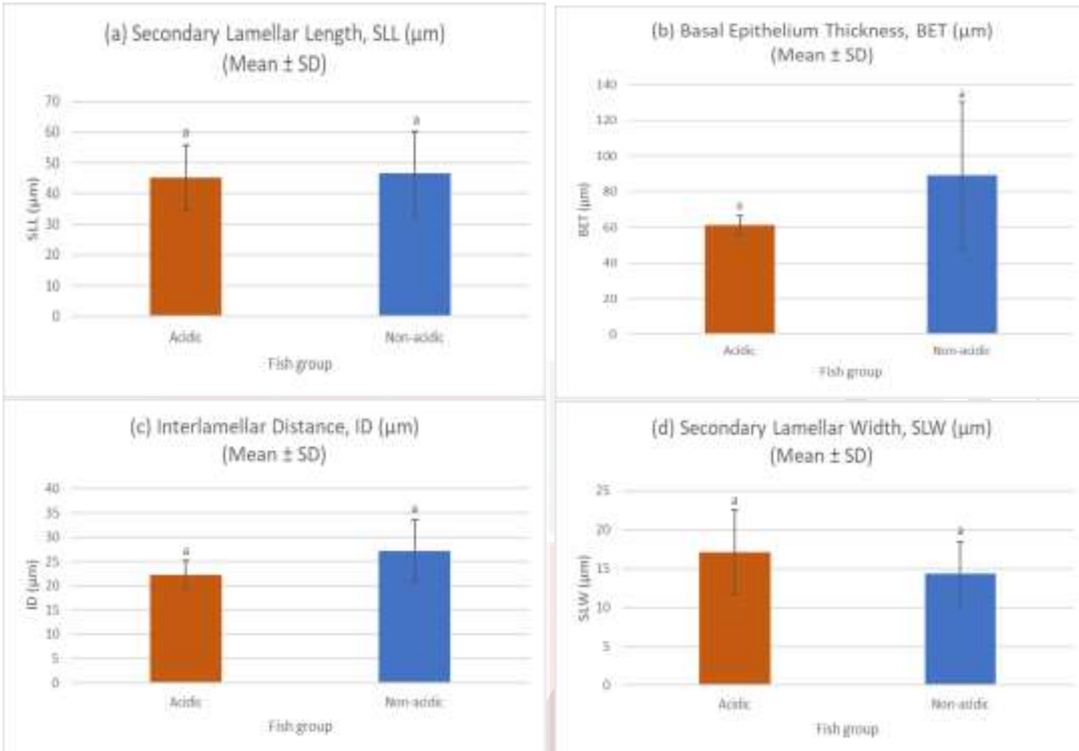


Figure 14: Photomicrograph of the gill morphometric measurements based on (a) Secondary Lamellar Width, (b) Secondary Lamellar Length, (c) Interlamellar Distance, and (d) Basal Epithelium Thickness.

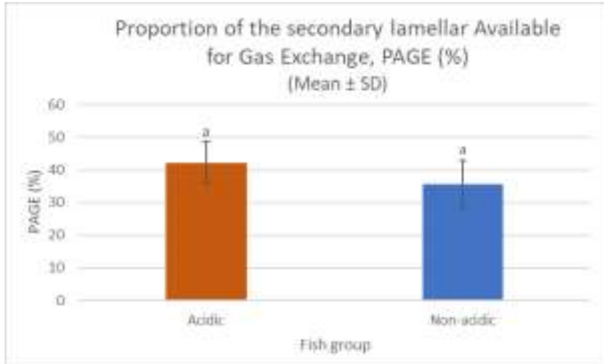
Based on gill morphometric analysis (refer to Figure 16) for *Anabas* fish in peat swamp group, the mean of secondary lamellar length (SLL) is slightly shorter, less basal epithelial thickness (BET), shorter interlamellar distance (ID), thicker secondary lamellar width (SLW) than those from the feeder canal group. However, from the independent sample T-test, there are no significant differences for SLL, BET, ID, and SLW between the two groups.



Data with different letter (a or b) between groups differ significantly (P < 0.05) (from Independent sample T-test).

Figure 15: Gill Morphometric Analysis through measurement of (a) Secondary Lamellar Length, (b) Basal Epithelium Thickness, (c) Interlamellar Distance, and (d) Secondary Lamellar Width (mean ± standard deviation).

The proportion of the secondary lamellar available for gas exchange (PAGE) (refer to Figure 17) was higher in peat swamp group (42.2%) compared to feeder canal group (35.6%). Thus, this shows that increase in number of secondary lamellar eventually increases the surface area available for gas/solute exchange. However, there are no significant differences of PAGE between the 2 groups based on the result of independent sample T-test performed.



Data with different letter (a or b) between groups differ significantly (P < 0.05) (from Independent sample T-test).

Figure 17: Proportion of the secondary lamellar Available for Gas Exchange between *Anabas* from peat swamp and feeder canal groups (mean ± standard deviation).

5.0 DISCUSSIONS

5.1 Water Chemistry

Peat swamp is usually referred as blackwater due to its accumulation of waterlogged sediments and acidic pH that prevents decaying plants material and thus, forming peat (United Nations Development Programme, 2006). From the results, North Peat swamp site have significantly low dissolved oxygen (5.06 mg/L) due to the presence of decaying plant materials and natural characteristic of peat swamp (Ng *et al*., 1994). The abundance of organic matters will result in natural dark colour appearance and acidity of the water pH commonly caused by reduction in photosynthesis of green algae (Irvine *et al*., 2013). This can be further explained where lower pH contributed by lower photosynthetic rate which results in higher carbon dioxide content and low in dissolved oxygen (Anugoolprasert *et al*., 2012). Meanwhile, water temperature obtained (27.4°C) maybe influenced by heat transfer from the atmosphere, water-air heat exchange (Kalinowska, 2019) or sunlight radiation as the recorded temperature was taken during daytime. This is because during daylight hours, water temperature is usually higher as a result of increasing air temperature (Irvine *et al*., 2013).

5.2 Gross Analysis

Both total body length and body weight of *Anabas testudineus* either in acidic (peat swamp site) or almost neutral environment (feeder canal) does not show any significant difference. Previous studies also show that the total body length and body weight of *Anabas testudineus* generally is within 8.5 to 14.5 cm and 10 to 15 g respectively regardless of the water conditions (Fitrani *et al*., 2011). Usually, the maximum length for this species without considering the sex of the fish is 25 cm

(Talwar and Jhingran, 1991) meanwhile, the common length would be 12.5 cm (Davidson, 1975). Physiologically, the weight increment is dependent on the length growth thus, expecting the body of *Anabas* fish to be symmetrical (Kordi, 2013).

According to the normal morphology of *Anabas* fish from earlier studies, the appearance of *Anabas* is dark to pale greenish, presence of longitudinal stripes on the ventral part of the head and dark sport on the opercular at the posterior margin (Herre, 1924). However, *Anabas testudineus* acquired from the swamp area in South Sumatra of Indonesia shows general dark gray to black colour appearance (Syafutri *et al*., 2018) which is similar to the finding of this study. However, this species is also categorized under black fish group (Muslim, 2007) due to the dark colouration characteristics of this species. Moreover, the slimy appearance which can be found from this study can be explained by the effects of acid toxicity (Mustapha and Atolagbe, 2018) which is also termed as mucous. Additionally, the mucus is defined as a viscous colloid which contains mucin that provides an innate immunity by continuous producing and sloughing process. Thus, preventing any adherence of microbes and helminths invasion on the surface of epithelium. Besides, mucins also contain proteins and enzymes that initiates innate immunity (Dash *et al*., 2018). Therefore, increase in mucous secretion in this study is probably an adaptive response to activate the mucosal immune system on the body surface and mucous membrane. On the other hand, the normal ganoid shaped of the scale of *Anabas* fish in peat swamp site appeared less well defined which might be due to the darker colouration that reduce the highlight of the scale. Normal *Anabas testudineus* would show large and regularly arranged scales (Herre, 1924).

Based on the macroscopic observation, there are no gross changes between the gills and kidneys between *Anabas testudineus* acquired from feeder canal and peat swamp site. Both gills showed normal structure of fish gills located on the lower head which extends to labyrinth via the first gill cavity. The gills are smaller and more rigid to ensure minimal collapse and ability to temporarily survive out of the water (Salleh *et al*., 2019). The rigidity of gills is due to the presence of cartilage support in primary lamellae that form from the back bone (Davenport and Matin, 2006). The appearance of gills which was reddening reddened indicating abundant of capillaries for direct oxygen uptake from air.

Meanwhile, both kidneys of *Anabas testudineus* showed normal structure and located in a retroperitoneal position, seated directly ventral to the vertebral column. Grossly, the kidney appears elongated, black or brown in colour and divided into anterior and posterior kidney. Most of the haematopoietic elements are from the anterior part while posterior part functioned as the excretory organ with nephrons as the functional unit.

Gross changes between the liver of *Anabas* fish acquired from peat swamp site and feeder canal is observed whereby the hepatic parenchyma of *Anabas testudineus* acquired from peat swamp site appeared peculiarly bright orange colour with pale yellow on its posterior part, and presence of rounded inferior margin with glistening capsule. This may indicate hepatic steatosis which is a reversible condition whereby large vacuoles of triglyceride fat accumulate in the liver cells, causing non-specific inflammation. Fish can employ some strategies such as physiological changes (lipid accumulation) or changes in feed preferences in order for it to survive in adverse conditions (Pastorino *et al*., 2019). As in *Anabas* fish, they require 28% proteins from their nutritious feed in order to elevate its survivability and growth rate (Suriansyah, 2012).

5.3 Histopathological Findings

Gills alterations of *Anabas testudineus* from the peat swamp site are similar to previous studies which are detachment of epithelium and hyperplasia of goblet cells. Besides, oedema is also obvious in this case which might be a consequent to detachment of epithelium. This is because, oedema occurred as a result of impaired osmoregulation which act as defense mechanism (Movahedinia *et al*., 2012) towards water acidification or irritants (Roberts, 2001). During severe oedema, gills epithelium detached entirely from primary or secondary lamellae concurrent with necrotic process that may lead to disturbance to osmotic regulatory or even death of the fish (Monteiro *et al*., 2008). However, in almost neutral pH condition, oedema and detachment of epithelium occurred most likely due to intoxication such as ammonia, chloride or even phenols (Satchell, 1984). This phenomenon helps to distance the pollutant-diffusion uptake which also acts as a protective mechanism (Mohavedinia *et al*., 2012). Meanwhile, with the presence of low dissolved oxygen and changes in pH or irritation by parasites or intoxicant may result in mucus proliferation as the primary reaction (Svobodova *et al*., 2005). Goblet cells eliminates pathogens and parasites or harmful materials from the surface of gills and neutralize physicochemical effects of most parasites with the presence of proteinaceous material (Strzyżewska *et al*., 2016).

Liver findings shows significant difference between the two groups based on hepatocyte swelling/hydropic degeneration and presence of intracytoplasmic lipid vacuoles. Hydropic vacuolation is considered to be the most common findings that can be found in fish from contaminated environment in relation to presence of chlorinated hydrocarbons (Stehr *et al*., 1998) which can also be found in peat soil (Kepple and Biester, 2003). The mechanism for this alteration initiated through fluid accumulation resulting in distention of hepatocyte cytoplasm or others may termed it as ballooning degeneration or cloudy swelling (Sther *et al*., 1998). Other studies also found the presence of cytoplasmic vacuolation due to excessive amount of glycogen after toxicity exposure (Nayak *et al*., 1996). Toxic effect study reveals that organophosphate pesticide trichlorfon which is used in agricultural activity such as paddy field area (Clémentine *et al*., 2019) may results in elevated lipid in hepatocytes (Xu *et al*., 2012). This finding helps to explain the presence of intracytoplasmic lipid vacuolation in fish from feeder canal area. However, in fish from peat swamp site, lipid vacuolation may be associated with survivability of fish in the wild whereby storage of glycogen under abundant availability of feed and synthesis of lipid during limitation of feed (He *et al*., 2015).

Most of the poor environmental quality can be indicated through formation of lesions in liver and kidneys of fish (Lidia et al. 2011). Renal lesions are good bioindicator to identify the toxic effects from contaminated water (Foulkes and Hammond 1975). From this study, pyknotic tubular epithelium and hydropic renal tubules are the significant alterations in kidney between fishes from the peat swamp and feeder canal groups. Presence of toxicants also show similar finding of pyknotic tubular epithelium (Rakhi *et al*., 2013). Meanwhile, hyaline droplets which is accumulations of intracytoplasmic protein absorbed from the glomerular filtrate and hyaline cast may arise from glomerulonephritis (Kashgarian, 1998) which mainly occur in antigen-antibody complex on glomerulus due to previous infection

(Ahmadmoradi *et al*., 2012).

5.4 Gill Morphometric Analysis

From the gill morphometric result, there are no significant differences of secondary lamellar length (SLL), Basal Epithelial Thickness (BET), Interlamellar Distance (ID), Secondary Lamellar Width (SLW) and the Proportion of the secondary lamellae Available for Gas Exchange (PAGE) between fishes from the peat swamp and feeder canal groups. This may depict that both group of *Anabas* fishes are surviving under different stressors, which can be either low pH or presence of toxicants, or irritants that may affect the gills surface area and cause gills alteration such as hyperplasia, proliferation of goblet cells etc. However, the result still shows that the acidic pH (peat swamp group) has short, flattened, and stumpy secondary lamellar, decrease in thickness of epithelium covering secondary, and increase in dimensions of secondary lamella which considered to increase efficiency of gills (Nero *et al*., 2005) for gaseous exchange, PAGE (42.2 %) compared to almost neutral pH (feeder canal group). The presence of narrow channels is therefore advantageous in systems where the flow is relatively rapid because it will reduce the total size of dead space between secondary lamellar (Hughes, 1966). Velocity of flow through secondary lamellar spaces will be high and insufficient time for diffusion and equilibrium between water and blood (Lloyd, 1961).

5.5 Future Recommendation

For future studies, it is recommended to increase sample size for both sites representing acidic and almost neutral pH water conditions. A challenge in capturing fish randomly from the wild is the absence of a guarantee that any fish would be caught successfully within a specified duration. With time and budget constraints faced by researches, gathering background information from the locals will help expedite the sample collection process hence increasing the sample size. Other than that, evaluation of other water quality parameters at peat swamp site should also be considered. This includes calcium ions, sodium ions and chloride ions since environmental calcium ions has been singled out as the most important environmental covariate in determining fish survival in acidic condition. Apart from these ions, aluminium is also considered to be the most significant metal ion with synergistic toxicity.

Other relevant studies related to this study include gross and histopathological changes on muscle and intestine of *Anabas testudineus* in peat swamp which can be compared to those from the feeder canal group. This has not been included in this study due to limitation of time and budget constraints. Additionally, gross and histopathological changes on gill, liver, and kidney of *Anabas testudineus* in alkaline pH condition can also be studied to identify possible lesions formed from an alkaline pH compared to the acidic pH condition.

6.0 CONCLUSION

There is presence of gross changes in the liver and histopathological changes of gills, liver and kidney between the *Anabas* fishes from the peat swamp (acidic) and feeder canal (almost neutral pH) groups. Therefore, this study failed to reject the null hypothesis on the absence of gross changes in the liver of *Anabas testudineus* that lives under extreme condition (peat swamp site) compared to those from natural environment (feeder canal) in North Selangor Peat Swamp forest. This study also failed to reject the null hypothesis for the absence of histopathological changes in gills, liver and kidneys of *Anabas testudineus* that lives under extreme condition (peat swamp site) compared to those from natural environment (feeder canal) in North Selangor Peat Swamp forest. Gill morphometric analysis revealed significant changes to the gills observed in the measurement of the secondary lamellar which was affected due to alterations for both acidic and almost neutral pH group. However, acidic (peat swamp) group has better oxygen efficiency in the aspect of higher proportion of the secondary lamellae available for gas exchange (PAGE) (42%) compared to almost neutral pH (feeder canal) group (35.6%). Although *Anabas* fish is considered to be extremophile, these changes show that acidic pH condition in peat swamp of the North Selangor Peat Swamp Forest has an impact on the gross and histopathological findings of *Anabas testudineus*.

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APPENDIX A

Table 4: Percentage (%) of Male and Female *Anabas testudineus* Fish Samples

		Frequency (N)	Percent (%)	Valid Percent (%)	Cumulative Percent (%)
Sex	Male	11	84.6	84.6	84.6
	Female	2	15.4	15.4	100.0
	Total	13	100.0	100.0	