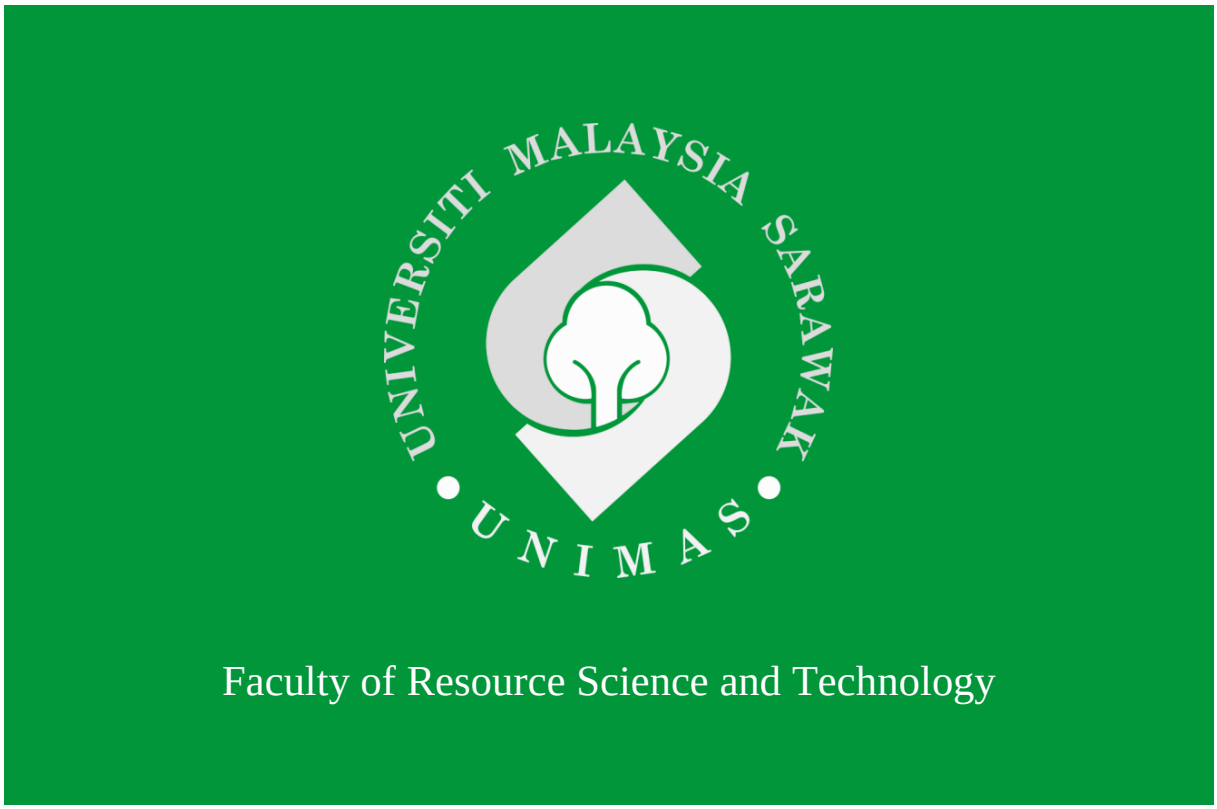

Effects of Selected Water Quality, Sulfide and Mercury on Juveniles *Barbonymus schwanenfeldii* and *Tor tambroides*

Azimah binti Apendi

Master of Science
2018

UNIVERSITI MALAYSIA SARAWAK

Grade: _____

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Final Year Project Report ☐

Masters ☑

PhD ☐

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Effects of Selected Water Quality, Sulfide and Mercury on Juveniles *Barbonymus schwanenfeldii* and *Tor tambroides*

Azimah binti Apendi

A thesis submitted

In fulfilment of the requirements for the degree of Master of Science

(Aquatic Science)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2018

DECLARATION

The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

_____

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ABSTRACT

Alterations of dissolved oxygen (DO), total suspended solids (TSS), temperature and pH could affect the growth and survival of fishes. Hydrogen sulfide and mercury, both under high concentrations are toxic and lethal to the aquatic organisms. In Sarawak, creation of new hydroelectric reservoirs results in changes in DO, TSS, temperature and pH with increasing sulfide and mercury. Freshwater fish species, *Barbonymus schwanenfeldii* and *Tor tambroides* have important economical values and act as a protein source to human. Both species require an optimum condition to survive in the nature and the changes in natural aquatic habitat due to anthropogenic activities lead to the reduced quantity and quality of these two indigenous species. This study aimed to determine the mortality and behavioral responses of both species to changes in water quality parameters, changes in the level of hydrogen sulfide and exposure to mercury in water and feed. In the water quality experiment, both species were exposed to 25 different combinations of temperature, TSS, pH and DO at three different levels, in 30 days' period. Response surface methodology was used to optimize the best set of condition for both species to survive. The results of this study show that avoidance when fed and longer feeding time were observed by both species in experiments with TSS higher than 1000 mg/L. Suspended solids in water reduced the visibility of fishes and affected their feeding activity. *B. schwanenfeldii* was predicted to survive the best under the combination of 27 °C, 0 mg/L TSS, 4.8 mg/L DO and pH 7.04 whereas the best set of condition for *T. tambroides* to survive are 23.9 °C, 0 mg/L TSS, 4.5 mg/L DO and pH 7.02. Sulfide tolerance was determined in 15 L containers with freshwater (100 mL/min) and sulfide stock solutions (5 mL/min) supplied. Methylene blue method was used to analyze the water for total sulfide concentration which was used to plot LC_{50}. Four behavioral responses were observed namely, huddling together, aquatic surface respiration,

loss of equilibrium and turned upside down. The responses were observed earlier for higher sulfide concentration compared to the lower sulfide concentrations. The LC_{50} of *B. schwanenfeldii* at 6 h was found to be 507.8 µg/L at 95% confidence level whereas for *T. tambroides* was 306.1 µg/L at 95% confidence level. Under this condition, the fish reached 100% mortality as early as 6 h at concentration 659 ± 39 µg/L and 50% mortality was earliest at 5 h at the same concentration. Lowering pH and dissolved oxygen levels were proven to aggravate sulfide toxicity. The adsorption of mercury through water was determined by exposing the fishes to mercury-spiked water while for adsorption through feed was done by feeding with mercury-spiked feed. Both experiments were carried out in 30 days. The concentrations of mercury in fish was determined by microwave assisted digestion then analyzed by mercury analyzer. In the mercury toxicity experiment, both *B. schwanenfeldii* and *T. tambroides* exhibited avoidance when fed and time taken to feed was also longer. Exposure of mercury through water exhibited higher survival rate for both species compared to feed exposure. However, the mercury concentration in both species' tissue were higher in water exposure than feed exposure experiment. In general, the experiments carried out show that changes in water quality parameters, hydrogen sulfide levels and exposure to mercury affected the mortality and behavioral responses of both species.

Keywords: Indigenous species, fish survival, behavioral response, sulfide tolerance, mercury toxicity.

***Kesan Kualiti Air Terpilih dan Merkuri terhadap Juvana* Barbonymus Schwanenfeldii *dan* Tor Tambroides**

ABSTRAK

Perubahan oksigen terlarut (DO), jumlah pepejal terampai (TSS), suhu dan pH boleh menjejaskan pertumbuhan dan kemandirian ikan. Kepekatan sulfida dan merkuri yang tinggi adalah toksik dan boleh membawa maut kepada kehidupan akuatik. Di Sarawak, pertambahan bilangan takungan hidroelektrik menyebabkan perubahan dalam aras DO, TSS, suhu dan pH sekaligus meningkatkan sulfida dan merkuri. Ikan air tawar, Barbonymus schwanenfeldii *dan* Tor tambroides *mempunyai nilai ekonomi yang penting dan merupakan sumber protein. Kedua-dua spesies memerlukan keadaan yang optimum untuk terus hidup, namun perubahan habitat akuatik semulajadi disebabkan aktiviti antropogenik telah menyebabkan pengurangan kuantiti dan kualiti spesies ini. Kajian ini bertujuan untuk menentukan tahap kematian dan tindakbalas tingkah laku kedua-dua spesies berdasarkan perubahan parameter kualiti air, perubahan sulfida dan pendedahan kepada merkuri dalam air dan makanan. Dalam eksperimen kualiti air, kedua-dua spesies didedahkan kepada 25 kombinasi suhu, TSS, pH dan DO pada tiga tahap berbeza, dalam tempoh 30 hari. Metodologi permukaan respons digunakan untuk mengoptimumkan keadaan terbaik untuk terus hidup. Keputusan kajian menunjukkan kedua-dua spesies mengelakkan makanan dan mengambil masa yang lebih lama untuk makan bila dalam keadaan TSS melebihi 1000 mg/L. Pepejal terampai dalam air mengurangkan penglihatan ikan dan menjejaskan aktiviti makan mereka.* B. schwanenfeldii *dijangka hidup dengan terbaik dalam kombinasi 27 ° C, 0 mg/L TSS, 4.8 mg/L DO dan pH 7.04 manakala keadaan terbaik untuk* T. tambroides *hidup adalah 23.9 ° C, 0 mg/L TSS, 4.5 mg/L DO dan pH 7.02. Toleransi terhadap sulfida ditentukan di dalam bekas 15 L dengan pengaliran air tawar 100 mL/min dan larutan stok*

sulfida 5 mL/min yang dibekalkan dengan kadar semakin meningkat. Kaedah metilen biru digunakan untuk menganalisis kepekatan jumlah sulfida yang digunakan untuk plot LC_{50}. Empat tindakbalas tingkah laku diperhatikan iaitu, berkumpul bersama, pernafasan permukaan akuatik, ketidakseimbangan dan terbalik. Tindakbalas yang lebih awal telah diperhatikan bagi kepekatan sulfida yang lebih tinggi. LC_{50} untuk B. schwanenfeldii *pada 6 jam berlaku pada kepekatan 507.8 µg/L manakala untuk* T. tambroides *ialah 306.1 µg/L pada tahap keyakinan 95%. Dalam keadaan ini, ikan mencapai kematian 100% seawal 6 jam pada kepekatan 659 ± 39 µg/L dan kematian 50% paling awal pada 5 jam pada kepekatan yang sama. Penurunan pH dan oksigen terlarut terbukti menambahkan ketoksikan sulfida. Pendedahan merkuri melalui air ditentukan dengan mendedahkan ikan kepada air yang ditambahkan merkuri manakala pendedahan melalui makanan dilakukan dengan memberi makanan yang telah dicampurkan merkuri. Kedua-dua eksperimen ini dijalankan dalam tempoh 30 hari. Kepekatan merkuri dalam ikan telah ditentukan oleh pencernaan mikrogelombang diikuti dengan analisa merkuri. Dalam eksperimen ketoksikan merkuri, kedua-dua spesies didapati mempamerkan pengelakan apabila diberi makan dan masa yang diambil untuk makan juga bertambah. Pendedahan kepada merkuri melalui air menghasilkan kadar kemandirian yang lebih tinggi bagi kedua-dua spesies berbanding pendedahan kepada makanan. Kepekatan merkuri di dalam tisu adalah lebih tinggi bagi eksperimen pendedahan kepada merkuri di dalam air daripada pendedahan melalui makanan bagi kedua-dua spesies. Secara amnya, semua eksperimen yang telah dijalankan menunjukkan perubahan parameter kualiti air, aras hidrogen sulfida dan pendedahan kepada merkuri mempengaruhi kematian dan respons tingkah laku kedua-dua spesies.*

***Kata kunci**: Spesies asli, kemandirian ikan, tindakbalas tingkahlaku, toleransi sulfida, ketoksikan merkuri.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
µg/L	Microgram per litre
µM	Micromolar
ANOVA	Analysis of variance
CCD	Central composite design
DO	Dissolved oxygen
FCR	Feed conversion ratio
Hg	Mercury
H_2S	Hydrogen sulfide
LC_{50}	Lethal concentration 50 percent
meHg	Methylmercury
mg/L	Milligram per litre
pH	Potential of hydrogen
PVC	Polyvinyl Chloride
RSM	Response surface methodology
SGR	Specific growth rate
SL	Standard length
SR	Survival rate
TL	Total length
TSS	Total suspended solids
WG	Weight gained

CHAPTER 1

INTRODUCTION

1.1 Research Background

Reservoir construction fragmented the rivers and become an obstacle for longitudinal exchanges such as nutrient recycling, water chemistry and migration (Brismar, 2004; Mc Cartney, 2009). This causes the reservoirs to become lentic thus enabling it to become stratified which can lead to changes in water chemistry, and accumulation of mercury and hydrogen sulfide. Changes in physical and chemical water quality parameters may influence the population of aquatic organisms by affecting the survival rate, growth, distribution and reproduction (Au *et al*., 2004). However, each species may respond differently to changes in water quality at different developmental stages (Ivoke *et al*., 2007).

Water quality parameters such as temperature, total suspended solids, pH and dissolved oxygen play an important role in maintaining an optimum habitat for aquatic organisms. The fluctuation of temperature can be influenced by the removal of trees along the banks, release of chemicals into water column and construction such as reservoir (Lessard and Hayes, 2002). Besides that, eutrophication and decaying process in an aquatic ecosystem will lead to a decline in the dissolved oxygen in the water column. An increase in total suspended solids is associated with the increase of turbidity in water which will affect the feeding rate of fish.

Decomposition of bacteria in the reservoir will convert inorganic mercury into toxic methylmercury. Methylation is carried out by sulfate-reducing bacteria which is usually present in deep and poorly-oxygenated water (Mason *et al*., 2000). Methylmercury is mainly accumulated in fish through the food chain and will harm human who consumes it. Hydrogen

sulfide is toxic to both human and aquatic life. It is introduced into the aquatic system through runoff from terrestrial (sewage treatment plants and manure-handling operations) or from the decaying process of organic materials in a confined place (Guidotti, 1996).

Following the impoundment of Batang Ai Hydroelectric Reservoir in 1985, Sarawak witnessed an increasing number of hydroelectric reservoir constructed namely Bakun Hydroelectric Reservoir in 2010 and Murum Hydroelectric Reservoir in 2014 (Nyanti *et al.,* 2012a; Ling *et al.,* 2013). Reservoirs caused fragmented rivers and become an obstacle for longitudinal exchanges such as nutrient recycling, water chemistry and migration (Brismar, 2004; Mc Cartney, 2009). This causes the reservoirs to become lentic and stratified, enriched with organic matter with depriving oxygen level that leads to production of hydrogen sulfide.

Barbonymus schwanenfeldii and *Tor tambroides* are two indigenous species in Sarawak freshwater (Ingram, 2005; Froese and Pauly, 2015). Both species are economically important as protein source and culturally important to the region (FAO, 2006). It is crucial to understand the behavior of these species including the survival, growth rate and behavioral characteristics under different water quality conditions and different concentrations of hydrogen sulfide and mercury.

Changes in water bodies such as the creation of reservoirs due to impoundment of rivers commonly lead to changes in water quality with the production of hydrogen sulfide and the mobilization of mercury. Some studies have reported on the increase of sulfide and mercury levels in the reservoirs due to the stratification. Increasing the levels of these two parameters could be related to the altered levels of DO, pH, TSS and temperatures in the water column. The deterioration of water quality levels affects fish physiology and growth (Wan Maznah *et al.,* 2014). In general, reservoirs struggle with resource degradation such as fish

assemblages and cause negative impacts on the local communities (Ambak and Jalal, 2006). Thus, the outcome of this study could be beneficial to Sarawak freshwater in both conservation and economic activities such as aquaculture.

What are the effects of these selected parameters on the survival of the juveniles? Were the juveniles able to tolerate altered levels of these parameters? Thus, the aim of this study was to investigate the effect of combined water quality parameters, changing levels of hydrogen sulfide and mercury exposure on the survival and mortality of selected indigenous fish species.

1.2 Specific Objectives

The objectives of this study were to:

i. determine the optimum combination of DO, pH, TSS and temperature for the survival and growth of *B. schwanenfeldii* and *T. tambroides,*
ii. determine the sulfide tolerance of both *B. schwanenfeldii* and *T. tambroides* under different sulfide concentrations with lowering DO and pH, and
iii. determine the mercury accumulation of juveniles *B. schwanenfeldii* and *T. tambroides* through exposure in water and feed.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of *Barbonymus schwanenfeldii* and *Tor tambroides*

Barbonymus schwanenfeldii or tinfoil barb is known locally as Tengadak. The species is a freshwater fish found in lakes and rivers and is distributed widely in Asia including Borneo, Sumatra and Peninsular Malaysia (Froese and Pauly, 2015). According to Mat Isa *et al*. (2012), *B. schwanenfeldii* breeds twice in 15 months and migrates upstream to release their eggs. The species is economically important as a protein source, and can be found abundantly in lakes, reservoirs, streams, canals and rivers (Mat Isa *et al*., 2012; Froese and Pauly, 2015). The fast reproduction rate of *B. schwanenfeldii* allows it to be cultured for example in Mekong River (FAO, 2006). Apart from that, it is also used as an aquarium fish and occasionally used as baits.

Mahseer *Tor tambroides* (Bleeker) locally known as Empurau or Kelah is culturally significant and possess economic importance in Sarawak (Ingram, 2005). The species is highly priced, valuable and sought after freshwater fish for both game and food fish in the region. It is known to inhabit clear and swift flowing waters with stony, pebbly or rocky bottoms and with availability of riverine fruits from growing trees by the banks (Ingram, 2005; Soon *et al*., 2014). *T. tambroides* has been known to migrate upstream during high flood period to spawn and downstream for feeding during the low-flow period (Ingram, 2005). However, development activities such as deforestation and reservoir construction as well as aquaculture practice and overfishing has threatened the availability of the species (Soon *et al.*, 2014). Such activities will deteriorate the environmental conditions, destroying the habitat of the fish. In Sarawak, its wild population have undergone declines in both abundance and distribution due to degradation of habitat and overfishing. Due to their

importance and value, a breeding program was established for conservation and aquaculture purposes by the Department of Agriculture Sarawak (Ingram *et al.*, 2007).

2.2 Influence of Total Suspended Solids (TSS) on Fish

In a hydroelectric reservoir, the regular fluctuation of large amount of water and reduction in sediment loads resulted in erosion of banks and floodplains where the sediment is deposited into the water (McCartney, 2009). High concentrations of total suspended solids (TSS) can lead to adverse effects such as clogging of gills, reduction in feeding rates, coughing, reduction in tolerance to disease and eventually death (Au *et al.*, 2004). Clogging of gills can lead to reduced oxygen supply as it interrupts with the respiration process. Suspended solids can cause variety of responses from fishes because many attributes of the physical environment are affected. High level of suspended solids lead to increase of turbidity of water thus reducing the visibility of food and reduction of light penetration which affect the primary production (Wood and Armitage, 1997). This explains the reduced feeding behavior in fish. However, some species such as *Alepes djedaba* and *Pranesus ogilbyi* thrive in turbid waters to reduce the risk of predation (Blaber and Blaber, 1980).

2.3 Influence of pH on Fish

Fluctuation of pH in water can lead to a diverse effect in aquatic organisms especially those of low tolerance. pH is important in the modulation of enzyme activity under physiological and pathological conditions in fish (Gorren *et al*., 1998). A study by El-Sherif and El Feky (2009) on the performance of tilapia *Oreochromis niloticus* in relation to pH shows that the decrease in pH caused the decrease in feeding rate thus leading to a fall in body weight. Both the lowest body weight and average feed consumption were recorded at pH 6 while the most optimum pH for the growth of tilapia is pH 8 and 9. The study on *Cyprinus carpio* L. by