



UNIVERSITI PUTRA MALAYSIA

**THE STUDY ON THE EFFECTS OF ENDOSULFAN ON FERTILIZATION,
HATCHING AND SURVIVAL RATE OF THE LEMON FIN BARB HYBRID
EGGS AND HATCHLINGS**

MUHAMMAD NUR FIKRI BIN MOHD NAZRI

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HATCHING AND SURVIVAL RATE OF THE LEMON FIN BARB HYBRID
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MUHAMMAD NUR FIKRI BIN MOHD NAZRI

**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
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DECEMBER 2022

CERTIFICATION

It is hereby certified that we have read this project entitled “The study on the effects of Endosulfan on fertilization, hatching and survival rate of the Lemon Fin Barb Hybrid eggs and hatchling”, by Muhammad Nur Fikri bin Mohd Nazri 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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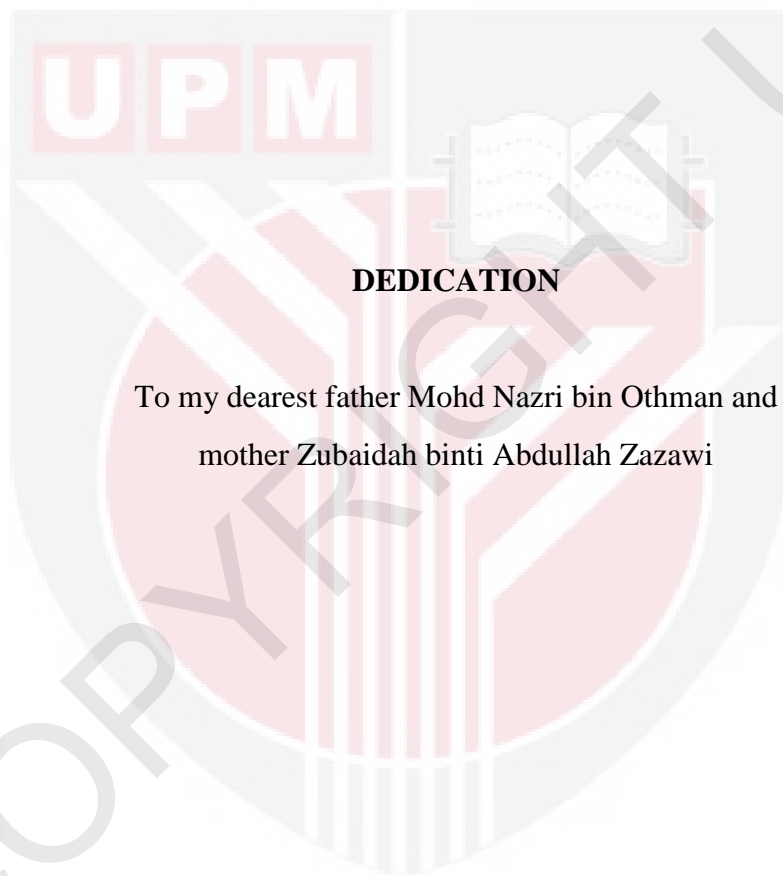
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DEDICATION

To my dearest father Mohd Nazri bin Othman and
mother Zubaidah binti Abdullah Zazawi

ACKNOWLEDGEMENT

First and foremost, my sincere gratitude goes to my supervisor Dr. Awang Hazmi Awang Junaidi for his guidance, patience, support and help in making this project a success. I also would like to thank him for his understanding in dealing with my problems throughout the study period.

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My highest appreciation goes to my beloved father, mother and sister for their continuous moral and financial supports. Not to forget my dearest friends Meiyappan Raja, Zhiling and Nazruddin for helping and accompanying me throughout my thick and thin FYP journey. Without them, surely this research could not be carried out perfectly.

Last but not least, special appreciation to the lovely LFBH that were used in this study. They are the 'unsung heroes' that make this study a memorable one. I hope that the findings in this project would be useful towards the improvement of aquaculture sector.

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LIST OF ABBREVIATIONS / SYMBOLS

ES	Endosulfan
et al.	And others
g	Grams
hr	Hour
kg	Kilogram
LFBH	Lemon Fin Barb Hybrid
ml	mililiter
Ppm	Parts per million
%	Percentage
>	More than
<	Less than
°C	Degree Celsius

ABSTRAK

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

KAJIAN TERHADAP KESAN ENDOSULFAN TERHADAP KADAR PERSENYEWAAN, PENETASAN DAN KELANGSUNGAN HIDUP HIBRID KERAI

LAMPAM

Disediakan oleh

FIKRI NAZRI

2022

Penyelia: Dr. Awang Hazmi Awang Junaidi

Penyelia bersama: Dr. Mazlina Mazlan, Dr Muhammad Fadhil Syukri Ismail

Aktiviti pertanian yang meluas memerlukan penggunaan racun perosak. Racun perosak boleh menyebabkan pencemaran sungai, serta memberi risiko kepada alam sekitar dan kesihatan hidupan akuatik. Endosulfan (ES) adalah merupakan racun serangga organoklorin yang masih digunakan secara haram walaupun penggunaannya telah dilarang di seluruh dunia. Dalam kajian ini, kesan ES ke atas kadar persenyawaan, penetasan dan kemandirian telur kacukan Kerai Lampam (*LFBH*; *Hypsibarbus wetmorei* × *Barbonymus schwanefeldii*) dan anak yang menetas telah dikaji. Pasangan Kerai Kunyit dan Lampam Jawa yang diperoleh daripada Unit Akuakultur, Fakulti Pertanian UPM, telah digunakan sebagai induk. Sperma dan telur

telah dituai dengan mengenakan tekanan ke atas perut ikan. Telur dan sperma telah dicampur dan diletakkan ke dalam tangki yang diisi dengan air yang dirawat dengan kepekatan ES yang berbeza; 0 ppm atau kawalan, 0.01 ppm, 0.1 ppm atau 1 ppm. Kadar persenyawaan dan penetasan dinilai secara in vitro pada 3j dan 18j selepas pendedahan terhadap ES (n=6/rawatan). Kadar kemandirian larva dinilai pada 24j, 48j dan 72j selepas penetasan (n=6/rawatan). Larva berumur 3 hari juga telah diambil sampel untuk menganalisis biometrik badan (iaitu, jumlah panjang dan ukuran kedalaman badan). ES memberi kesan yang ketara terhadap kadar persenyawaan dan penetasan pada 1 PPM (masing-masing $p < 0.05$; $p = 0.041$ and $p = 0.001$). Kadar persenyawaan dan kadar penetasan telur yang dirawat dengan 1 ppm ES (masing-masing $44.24 \pm 4.6\%$ and $18.54 \pm 2.8\%$), adalah lebih rendah daripada nilai kawalan (masing-masing $63.35 \pm 5.8\%$ dan $46.76 \pm 1.3\%$). Kesan rawatan dan masa ke atas kadar kemandirian larva juga adalah ketara ($p < 0.05$; $p = 0.019$ and $p = 0.09$, masing-masing). Analisis biometri badan menunjukkan tiada perbezaan yang signifikan ($p > 0.05$) dalam jumlah panjang dan lebar larva, antara rawatan. Kesimpulannya, pendedahan ES memberikan kesan negatif terhadap perkembangan awal telur LFBH dan kemandirian ragi. Penemuan awal ini menyediakan data berharga untuk kajian masa depan tentang toksikologi ES pada spesies ikan air tawar lain di Malaysia.

Kata kunci: hibrid kerai lampam, endosulfan, penetasan, persenyawaan, kelangsungan hidup

ABSTRACT

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

**THE STUDY ON THE EFFECTS OF ENDOSULFAN ON FERTILIZATION,
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By

FIKRI NAZRI

2022

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Co-supervisor: Dr. Mazlina Mazlan, Dr Muhammad Fadhil Syukri Ismail

Extensive agriculture activities demand the use of pesticides. Pesticides could pollute the rivers and streams, possessed an environmental and health risk to aquatic life. Endosulfan (ES), an organochlorine insecticide, is still illegally used, despite the worldwide ban. In this study, the effect of ES on the fertilization, hatching and survival rate of the lemon fin barb hybrid (LFBH); (*Hypsibarbus wetmorei* × *Barbonymus schwanenfeldii*) eggs and hatchlings were examined. A pair of LFBH, was used as the broodstock. The sperm and eggs, harvested *via* the stripping method, were mixed and treated with different concentrations of ES; 0 ppm or control, 0.01 ppm, 0.1 ppm or 1 ppm. The fertilization and hatching rate were evaluated *in vitro* at 3 hr and 18 hr post-exposure, respectively (n=6/treatment). The survival rate of the larvae was assessed at 24 hr, 48 hr and 72 hr post-hatching (n=6/treatment). The LFBH eggs treated with 1

ppm ES demonstrated lower fertilization ($44.24 \pm 4.6\%$) and hatching rate ($18.54 \pm 2.8\%$) ($p < 0.05$), than the control ($63.35 \pm 5.8\%$ and $46.76 \pm 1.3\%$, respectively). The main effect of treatment and time on the survival rate of the larvae was also significant ($p < 0.05$; $p = 0.019$ and $p = 0.09$, respectively) within three days' post-hatchlings. Overall, ES exposures display a detrimental effect on the early development and survival of the LFBH eggs and hatchlings. The findings in this preliminary study provide valuable insights for future studies on the toxicology of ES on other native freshwater fish species in Malaysia.

Keywords: lemon fin barb hybrid, endosulfan, hatching, fertilization, survival

1.0 INTRODUCTION

Agriculture is an important sector of economy in Malaysia's, contributed approximately 7.4% of annual national GDP (SRD, 2022). Since the 1900, commodity plants such as rubber, oil palm and cocoa has been the main exports. Currently, Malaysia is one of the major producers of palm oil in the world and the plantation alone covers over 5 million hectares of land (MPOB, 2011). Approximately 60% of the oil palm plantations are concentrated in the Malaysian Borneo states. In Peninsular Malaysia, Pahang top the list being the state with largest oil palm plantation by area (hectares); 12.8 % (Kushairi and Ahmad, 2017).

Extensive agriculture activities demand the use of fertilizers and pesticides, to ensure optimum yields and control of pests. According to Muller (2022), ~44 thousand tonnes of pesticides were used in Malaysia as of 2018, with the pesticide industry worth of RM 1.5 billion in 2019. Although highly regulated, the overuse of pesticides remains a major issue that need to be addressed. According to Pimentel and Burgess (2011), only less than 0.1 percent of pesticides applied reached the target pest, while the rest went into the environment, mostly into the water resources through leaching and runoff. The presence of pesticide residues in Malaysian rivers (Aminuddin et al, 1996; Leong et al. 2007; Haron et al. 2015) is alarming, and could possesses an environmental and health risks.

Endosulfan (ES), belongs to the organochlorine group pesticide, have been used extensively in many parts of the world (Weber et al., 2010), before it has been banned globally in 2012. The threats to humans and other living organisms include

neurotoxicity, infertility, respiratory depression, organ failures and behavioural change (Amizadeh et al., 2011). In Malaysia, ES was detected in several Malaysian rivers (Leong et al., 2007), evidenced the illegal used of ES pesticides, despite the banned since 2005 (Rengam, 2009).

Pahang River provides the environment suitable for the breeding and growth of freshwater fish. However, the deterioration of water quality due to human activities (i.e., oil palm plantation and mining) along the river and its tributaries (Mardiana-Jansar and Ismail 2015) exposed a great threat to the aquaculture industry in the long term. To date, the data on the level of pesticides residues in Pahang River are scarce. The presence of pesticide residues like ES could potentially disrupt the hormonal and reproductive functions of freshwater fish, as well as stunted their growth, resulting a delay to reach the market size or weight.

In this study, the effects of ES on the early life stages of Lemon Fin Barb Hybrid (LFBH) were studied, with special emphasis on their fertilization and hatching rates. The survival and development of the larvae for the first 3 days' post-hatching was also evaluated. LFBH is a crossbreed between the, *Hypsibarbus wetmorei* and *Borbonymus gonionotus* (Zakaria et al., 2018). It was first produce in 2012 with the special traits include a good taste and attractive exterior traits. Therefore, LFBH is a good modal to study the effect of ES to the native freshwater fish.

2.0 LITERATURE REVIEW

2.1 Aquaculture industry

Aquaculture is one of the important contributors to Malaysian economy since the past decades. Among the goals of aquaculture industry is to improve the nation's food security, raise income and export earnings, maximise producer income, and to reduce poverty (Roslina, 2018). According to DOF, freshwater fish production has increased to 8.9% from 2020 to 2021. In nations with high meat consumption such as Malaysia, fish could be utilize as an alternative protein source (de Boer et al. 2020). According to Department of Fisheries, Malaysia (DOF,2020), about 1.85 mt of fish were produced through the aquaculture capture and inland fisheries with the estimate value at USD 3.3 billion (RM 14.5 billion). Based on the study, about 92% of the country's self-sufficiency level are come from agriculture sector. Among them, the aquaculture industry in Malaysia generates 391,000 mt with a value estimated at around USD 724. (RM 3.1 billion) (Azra et al., 2021).

2.2 Hybridization of fish

Hybridization is the process an animal breeding with an individual of another species. It is one of the approach to maximize the aquaculture production of the freshwater and marine fish (Rahman et al., 2018). The goal of the hybridization is to induce hybrid vigour or positive heterosis, in which the desirable traits from each species are expressed and pass to the next generation. Generally, the positive traits such as disease resistance, high growth rate, and good meat quality are targeted (Bartley et al., 2000). This characteristic is crucial to ensure optimal quality and production of aquaculture.

Hybridization of fish programme has been successfully implemented in many part of world including countries like the United States, Thailand, Venezuela and Israel. Hybrid striped bass, clarias catfish, characids and hybrid tilapia are among the hybrid fish that have been produce for aquaculture and stocking programme. In Malaysia, several hybridization programme conducted by scientist from several universities have successfully produced hybrid fish and this include hybrid grouper, red hybrid tilapia and LFBH.

2.3 Lemon Fin Barb Hybrid

LFBH or locally known as “Kerai Lampam” is a cross-breed between *Barbonymus gonionotus* (Kerai Kunyit) and *Hypsibarbus wetmorei* (Lampam Jawa) (Zakaria et al., 2018). They are two closely related freshwater fish found in Southeast Asia. These fish are omnivorous cyprinids, that are native to Southeast Asia, and can be found throughout the Mekong, Chao Phraya, Pahang rivers basins, as well as in the island of Sumatra and Java. Lemon fin barb is a non-aggressive gorgeous species which features metallic golden scales in the shape of diamonds with colorful fin tips (Fishbase, 2012).

This species is usually caught wild and fresh to be sold as ornamental or meat fish as they are mostly wildlife.

According to study, *B. gonionotus* can breed and grow easily in captivity whereas *H. wetmorei* possess attractive exterior traits and claim to have a delicious taste (Chaudhary et al., 2009; Romano et al., 2017). After looking into this characteristics, Malaysian Department of Fisheries find a values by crossing the male of Lemon fin barb to the female of Jawa barb to create a hybrid LFBH in 2004. The interest for this

hybrid in aquaculture sector has then showed an increasing trend as the hybrid has both fast growth rate and tasty meat contributed by this two species (Zakaria et al., 2018). LFBH are said can reach to the market size (500g – 600g) only in five to six months.

2.4 Pesticides

In agricultural settings, pesticides are employed to control the proliferation of pests. Typically, these pesticides are toxic to aquatic life and other non-target animals (Pimentel, 1995). The three primary categories of pesticides are carbamates, organochlorines, and organophosphorus (Pimentel, 1995; Van Dyk and Pletschke, 2011). Organochlorines is the most prevalent pesticides found in the environment (i.e, water, sediments, atmospheric air, and the biotic).

Organochlorine pesticides was seen as a safe and reliable compound for pest control for the past six decades until a growing number of problems connected to their use emerged. According to Parameswari et al. (2020), less than 1% of pesticides applied reaches the targeted pest while the remaining (>90%) enters the different components of the environment to contaminate water, soil, food, air, forage, feed and other commodities.

It has been reported about 385 million cases of acute pesticide poisoning have been reported worldwide, causing around 11,000 deaths (Boedeker et al., 2020). Exposure to pesticide could lead to acute and chronic adverse health effects. Rashes, itchy eyes, blindness, blisters, nausea, diarrhoea, disorientation, and even death are among the health impacts related to short-term pesticide exposure. Meanwhile, developmental

abnormalities neurological toxicity, cancer and disruption of the endocrine system are among the impact of long-term exposure. (Parameswari et al., 2020). Due to the aforementioned negative effects, many pesticides have been gradually phased out over the years, globally. In Malaysia, the Malaysian Ministry of Agriculture has prohibited the application of 40 different types of pesticides. Among them are chlorinated pesticides, including alachlor, aldrin, chlordane, DDT, dieldrin, HCH isomers, heptachlor and endosulfan (Pauzi et al., 2015).

2.5 Endosulfan

ES is a broad-spectrum organochlorine pesticide that has been used in agriculture to eradicate insects and mites from crops, such as ornamental trees, shrubs, and vines as well as fruits, vegetables, and cereals. Despite the benefits, ES creates problems due to its persistence in the environment where its residue may stay in soil for several years before breaking down (Rosenfeld and Feng, 2011). The hydrophobic and semi-volatile nature of ES, make it prone to enter the natural streams, soil and air leading to bioaccumulation in a variety of living organisms, including human (Jayaraj et al., 2016). The United States Environmental Protection Agency has considered ES as an 'extremely dangerous' chemical and hazardous to many species of living organism (Marziyeh and Ghasem, 2011). It possesses a significant short- and long-term influence on human health and caused hundreds of fatalities globally. Several cases of ES toxicity has been reported from different part of the world. One of the tragic examples is the one that happen in Kerala, India where at least 4000 people were died (James and Emmanuel, 2021). The propensity of ES to travel across great distances in

the environment, along with its negative impacts, require the necessity for coordinated worldwide action. Thus, it was phase out globally since 2018.

In Malaysia, enforcement of the ban on the usage of ES has been implemented since 2005 (Rengam, 2009). Despite the banned, people can still find a way to purchase them illegally in the online and black market. The illegal use of ES was evidence by the discovery of ES residues in several rivers in Selangor and tap water in Brinchang, Pahang. compounds namely aldrin, ES II, endrin ketone and 4,4'-DDE in the tap water of Berinchang town (Pauzi et al., 2015).

This is very worrying because it endangers aquatic life, marine ecosystems, environment and humans. In addition, ES can be also found in water and can persist for several weeks to months, depending on the chemical properties of the compound.

2.6 The effect of endosulfan

ES is considered as 'extremely dangerous' chemical and hazardous to many species of living organism. by the United States Environmental Protection Agency (Marziyeh and Ghasem, 2011). It has a significant short- and long-term influence on human health and caused hundreds of fatalities globally Exposure to ES could be acquired through the contaminated soils, air, and water (Marziyeh and Ghasem, 2011; Amizadeh and Saryazdi, 2011). The propensity of ES to travel across great distances in the environment, along with its negative impacts, require the necessity for coordinated worldwide action. ES has been banned worldwide since 2008. However, it has been banned much earlier in Malaysia, since 2005 (Rengam, 2009).

2.6.1 The effects on endocrine functions

At the early stage of development, fish are more prone to have an endocrine disrupting effects. Exposure of ES can lead to reproductive and developmental effects on the non-target animals. According to reports, ES exposure can cause reduce cortisol production in fish (EPA, 2002). Simultaneously, it can suppress androgen and oestrogen receptors, which can affect the metabolism of sex hormone. ES role as an endocrine disruptor is achieved by inducing the production of the vitellogenin protein. This will trigger the release of E2 from the gonads and disrupts the interaction between oestrogen and its receptors (Andersen et al., 1999).

2.6.2 The effect on development

One of the effects of ES is it may lead to reproductive dysfunction either in the male or female fish (Chakravorty et al. 1992; Rajakumar et al. 2012; Chakrabarty et al. 2012). ES can disturb the immunity and body defense of aquatic animal due to the inhibition of vitellogenesis (Chakravorty et al., 1992). Vitellogenin, a female-specific reproductive protein, plays an important role in providing the nutrients for developing embryo and involve in the immune defence against microorganisms like bacteria and viruses (Zhang et al., 2015). Slow ovarian activity, abnormal ovarian wall thickness, disruption of oogenesis are other effects of ES on female reproduction (Pandey, 1988).

In male fish, ES was found to impair testis development, particularly disrupting the spermatogenesis, as well as reduce plasma testosterone, sperm motility and sperm count (Choudhary and Joshi, 2003; Rajakumar et al. 2012; Balasubramani and Pandian 2008). Other than that ES is also known to cause morphological changes to fish vital organs at acute exposure (Ganeshwade et al., 2012). Another study by Saaristo et al.

(2018) also stated that repeated sub-lethal exposure will lower the organism's resistance to disease hence causing it to have lower survival rate in harsh environment.

2.6.3 The effects on behaviour

The most reliable indicator of the toxic effects of ES is the behavioural changes (Ullah et al. 2005; Bretaud et al. 2000; Pereira et al. 2012). Feeding habits, swimming pattern, competition, predation and reproduction are among the aspects that are commonly assessed in the toxicity event. According to (Pereira et al., (2012), exposure to of ES could impaired the movement of fish, based on their observation in the Zebra fish (*Durio rerio*). Basically, it reduces the exploratory ability and mean speed of the fish, accompany by abnormal swimming pattern. ES decrease the mobility in fish by interrupting the acetylcholinesterase activity, which are important during impulse transmission (Bretaud et al., 2000).

3.0 MATERIALS AND METHODS

3.1 Experimental animals.

A pair of matured Lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂), weighing from ~250 g, were used as a brood stock. The fish were obtained from the UPM Fish Hatchery Centre. The female and male fish was injected with 0.6 and 0.3 ml/kg Ovaprim (Syndel, Wastern Chemical Inc., MIF 900 - 001 and Canada), respectively prior to clutching, to induce final maturation. The eggs and sperms were gently clutched after 6 hr of injection. The sperms and eggs were gently mixed in a bowl using chicken's feather. The mixture was exposed to different concentration of ES (i.e., 0 ppm or control, 0.01 ppm, 0.1 ppm, 1 ppm). The mixture was transferred into a treatment tank (one tank/treatment) with the ambient temperature kept at 23.0 – 25.0 °C. A schematic overview of the experimental design was shown in Figure 1.

3.2 Fertilization and hatching rate.

The eggs in the treatment tank were randomly selected and transferred into the petri dish by using a dropper. The fertilization and hatching rate evaluation were performed using 6 replicates per each treatment (n=6). The eggs were continuously suspended in their respective treatment solution. The fertilization and hatching was evaluated under stereomicroscope (Olympus 1.2 X 10) and Dyno-Eye (AM7025X) at 3 hr and 8 hr post-exposure of ES. The unfertilized LFBH eggs was characterized by disintegrated and sunk, with cloudy appearance. Meanwhile, the fertilized eggs were evidenced by

the blastocyst and organogenesis. The formula for the calculation of fertilization and hatching rate were shown in Figure 2 (a) and (b).

3.3 Survival rate

The survival rate of LFBH larvae was evaluated at 24 hr, 48 hr and 72 hr post-hatching. The survival rate assessment was performed using 6 replicates per each treatment (n=6). Live larvae were observed and counted by naked eye, depicted by their motility. The formula for the calculation of survival rate was in Figure 2 (c).

3.4 Body biometry

Body biometry of the larvae was measured and recorded at 72 hr post-hatchings. Ten larvae (n=10/treatment) was randomly selected, and their total length and body depth were measured and recorded, with the aid of the stereomicroscope (Olympus 1.2 X 10) and Dyno-Eye (AM7025X, DinoCapture 2.0). The total length indicated the measurement made from the tip of the snout to the tip of the longer lobe of the caudal fin. Meanwhile, the body depth indicated the measurement made from the maximum vertical distance between dorsal and ventral margin of the larvae body (Figure 3).

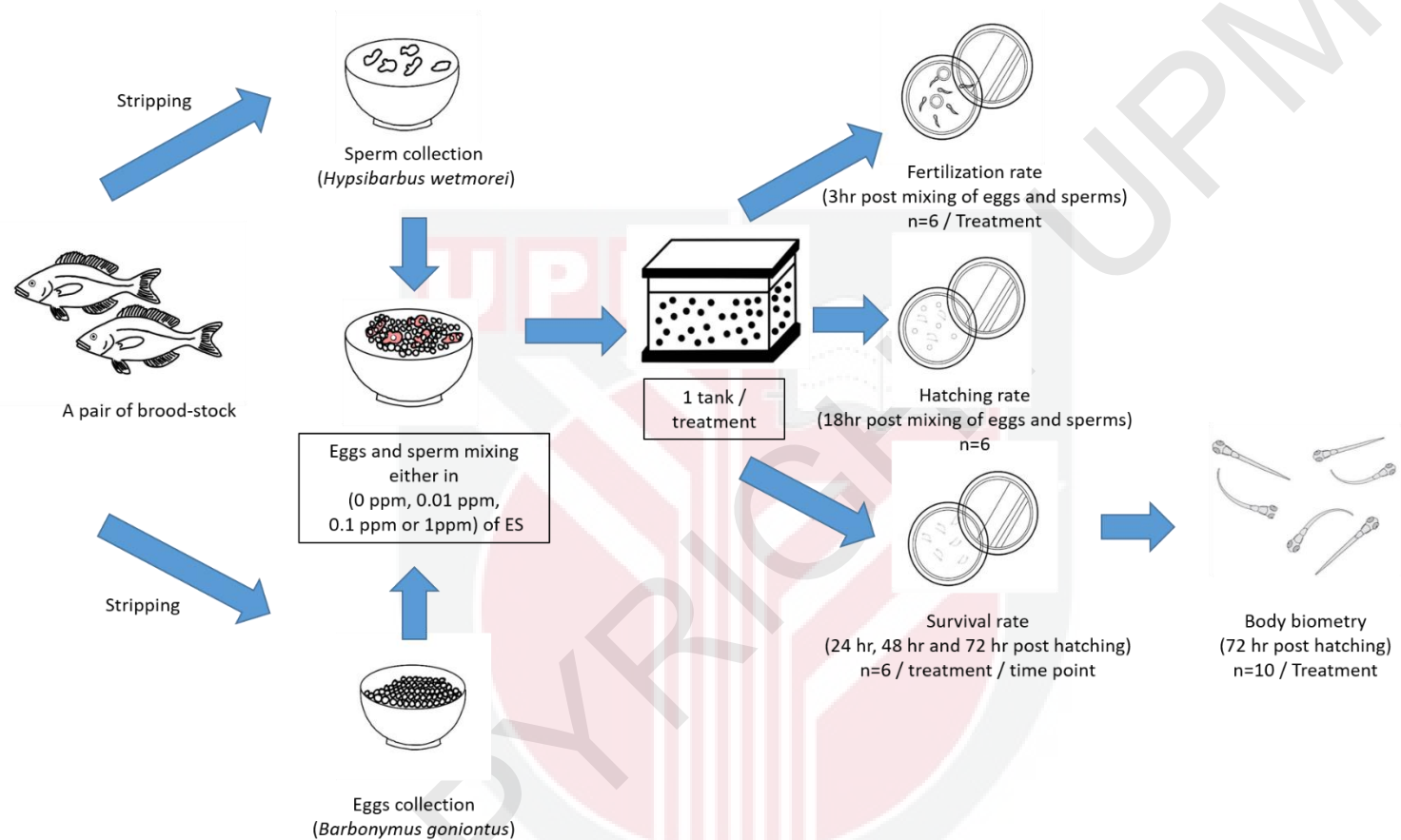


Fig.1 Schematic overview of the experimental design. The eggs and sperms were obtained from a pair of brood stock via a stripping method: male is of silver barb (*Barbonymus gonionotus*) and female is of lemon fin barb (*Hypsibarbus wetmorei*). Eggs and sperms were mixed and kept in water composed of either 0 ppm (control), 0.01 ppm, 0.1 ppm and 1 ppm of endosulfan (ES) and transferred to a pool tank (1 tank/treatment). The fertilization and hatching rate of the eggs was assessed and recorded at 3 hr and 18 hr post exposure, respectively. The survival rate was evaluated at 24 hr, 48 hr and 72 hr of post exposure to ES. Body biometry of LFBH larvae was also taken 72 hr post hatching, by measurement of the total length and the body depth.

$$\text{a) Fertilization rate (\%)} : \frac{\text{Number of fertilized eggs}}{\text{Total no of eggs}} \times 100$$

$$\text{b) Hatching rate (\%)} : \frac{\text{Number of viable larvae}}{\text{Total no of eggs}} \times 100$$

$$\text{c) Survival rate (\%)} : \frac{\text{Number of larvae survived}}{\text{Total no of larvae hatched}} \times 100$$

Fig. 2 The formula for calculation of the (a) fertilization rate, (b) hatching rate of Lemon Fin Barb Hybrid eggs and (c) survival rate of the hatchlings after exposure to Endosulfan.

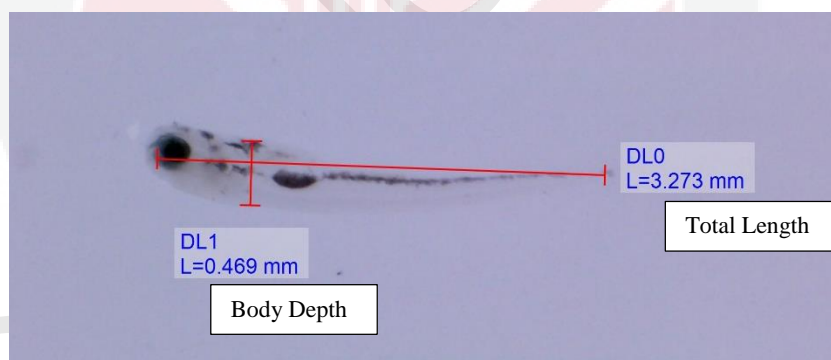


Fig. 3 Representative image showing the body biometry evaluation of the LFBH larvae. The total length was measured from the tip of the snout to the tip of the longer lobe of the caudal fin while body depth measured from the maximum vertical distance between dorsal and ventral margin of the fish body.

4.0 RESULTS

4.1 Fertilization and hatching rate

In general, the fertilization and hatching rate demonstrated a declining trend over dosage (*i.e.*, control > 0.1 ppm > 0.1 ppm > 1 ppm). The fertilization rate of eggs exposed to 1 ppm ES ($44.24 \pm 4.6\%$) differed significantly ($p < 0.05$; $p = 0.041$) compared to the control ($63.35 \pm 5.8\%$) (Figure 4.1a). Similarly, the hatching rate was also significantly lower ($p < 0.01$) in the group exposed to 1 ppm ES ($18.54 \pm 2.8\%$) compared to the control ($46.76 \pm 1.3\%$), and the group exposed to 0.01 ($42.21 \pm 5.6\%$) and 0.1 pm ES (38.26 ± 8.4) (fig 4.1b).

4.2 Development of embryo

The fertilization and embryonic development of the fertilized LFBH eggs was monitored over time and depicted in Figure 4.1 (a-f). Unfertilized LFBH eggs was characterized by disintegrated and sunk, with cloudy appearance (Figure 4.1a). Meanwhile, the fertilized eggs were evidenced by the blastocyst and organogenesis. Within 30 minutes of fertilization, fertilized eggs had a spot (blastodisc) on one pole and were easily identifiable with the naked eye. During organogenesis, the head and tail end of the embryo was differentiated. The embryo was elongated and encircled the yolk materials. Both tail and head ends were clearly differentiated.

4.3 Survival rate

There was a significant effect of treatment on the survival rate of larvae ($p < 0.05$; $p = 0.019$). However, there was no significant effect observed on individual ES treatment upon post hoc-test. The effect of time on the survival rate was also significant ($p < 0.05$; $p = 0.010$) for the control group. In the control group, the survival rate of LFBH larvae

was significantly lower ($p < 0.05$; $p = 0.010$) at 72 hr post-hatching ($83.71 \pm 2.7\%$) than at 24 hr ($92.20 \pm 0.77\%$).

4.4 Body biometry

There was no significant difference in the total length ($p > 0.05$; $p = 0.249$) and width ($p > 0.05$; $p = 0.155$) of LFBH larvae exposed to different dosages of ES at 72 hr post-hatching (Figure 7 b and 7c, respectively). In general, the total length and width of LFBH larvae was approximately (3.34 ± 0.15) and (0.46 ± 0.04), respectively.

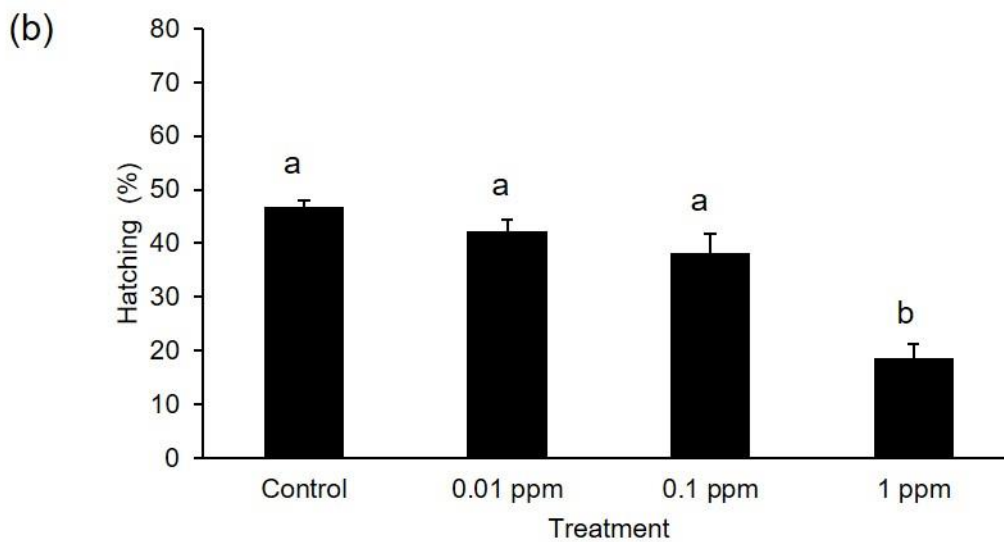
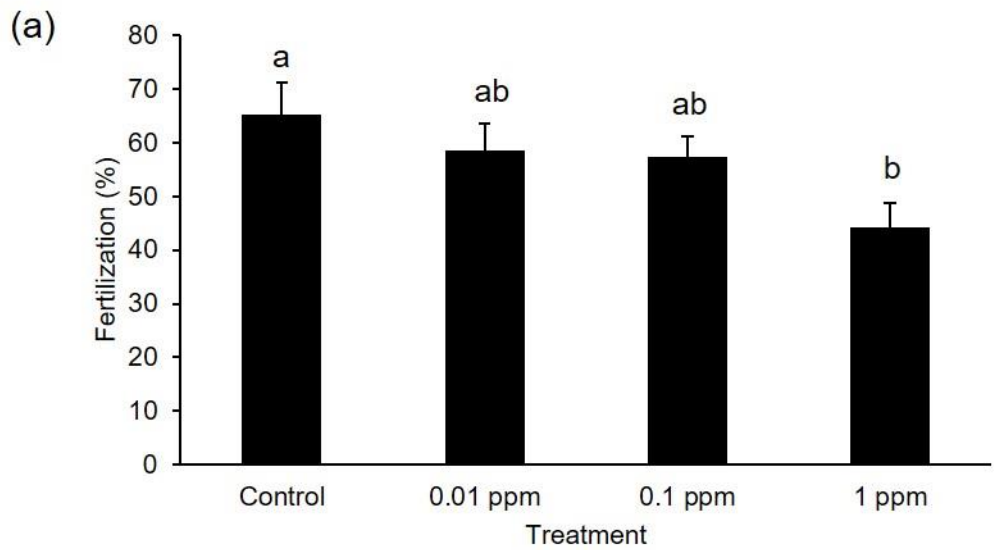


Fig. 4 The (a) fertilization and (b) hatching rate of Lemon Fin Barb Hybrid exposed to different concentration of endosulfan (ES) (i.e., either 0.01, 0.1, or 1 ppm). The control group was placed in the environment absence of ES. The fertilization and hatching rate were evaluated at 3 hr and 18 hr post sperm and egg mixing, respectively. Data are mean \pm S.E.M. Data with difference letters (a and b) differs significantly ($p < 0.05$).

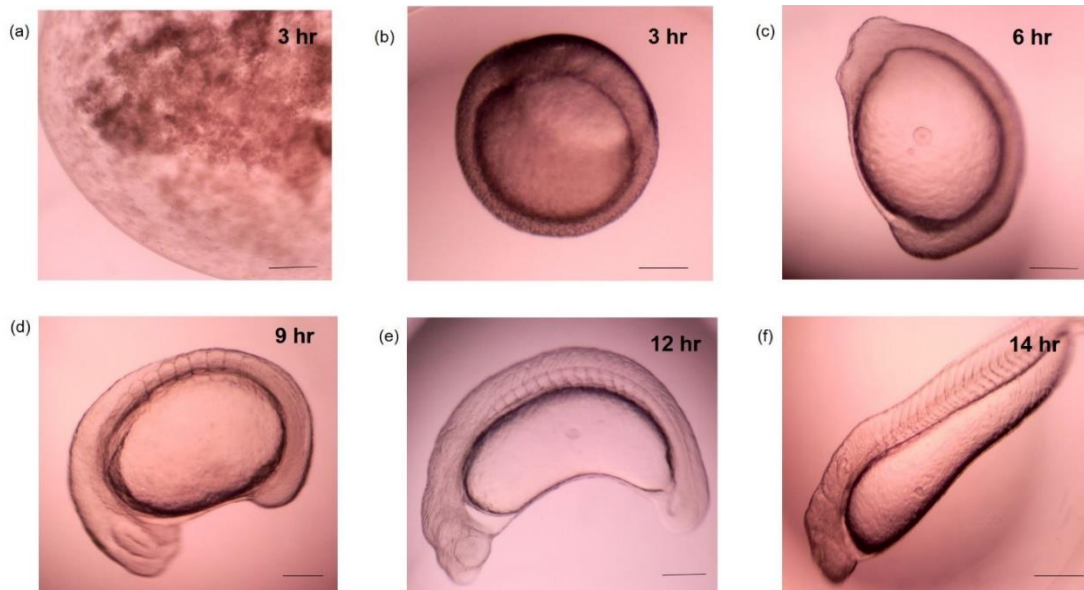


Fig. 5 The development of Lemon Fin Barb Hybrid embryo over time. (a) Representative image of unfertilized egg. The egg was disintegrated and sunk with cloudy appearance. (b-f) Representative images showing the fertilized and embryo development. (b) Late gastrula stage (c) Blastophere closure stage. (d) Embryo with somite and optic primordium, optic vesicle and olfactory placode and tail bud. (e) Advance stage showing the presence of otic capsule, tail vesicle and lense formation. (f) Just before hatching, the embryo became straighten, losing the C shape, with otolith appearance. (Scale bar = 200 μm).

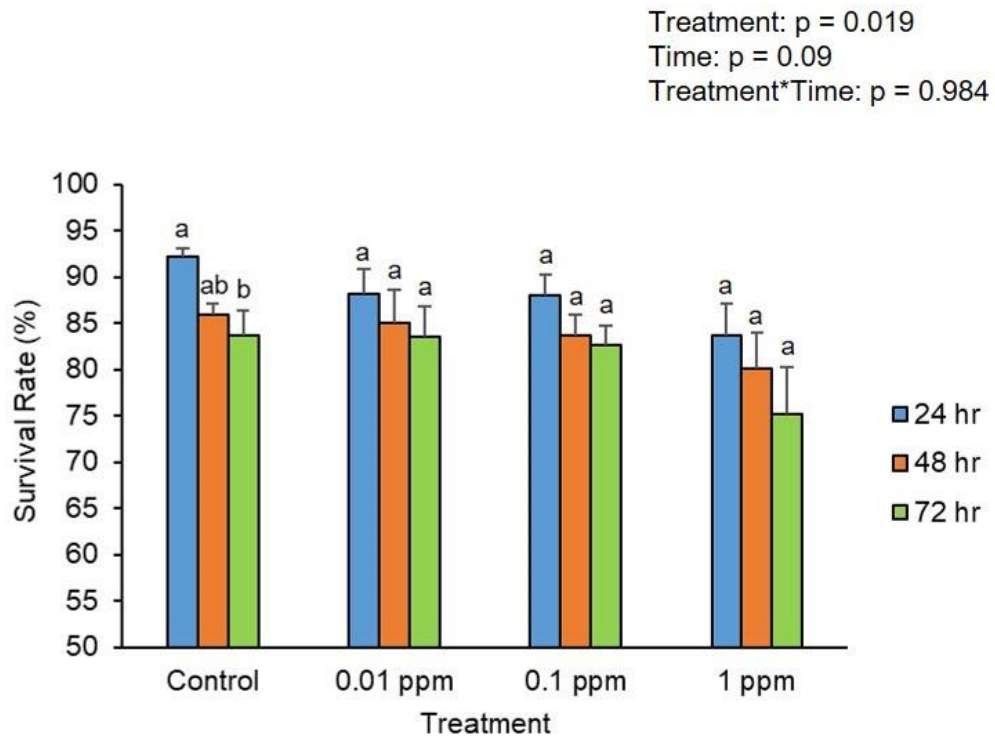


Fig. 6 Survival rate of Lemon Fin Barb Hybrid larvae, exposed to endosulfan (either at 0.01, 0.1 and 1 ppm) at 24 hr, 48 hr, 72hr post-hatching. The control group was placed in the environment absence of endosulfan. Data with are mean \pm S.E.M.

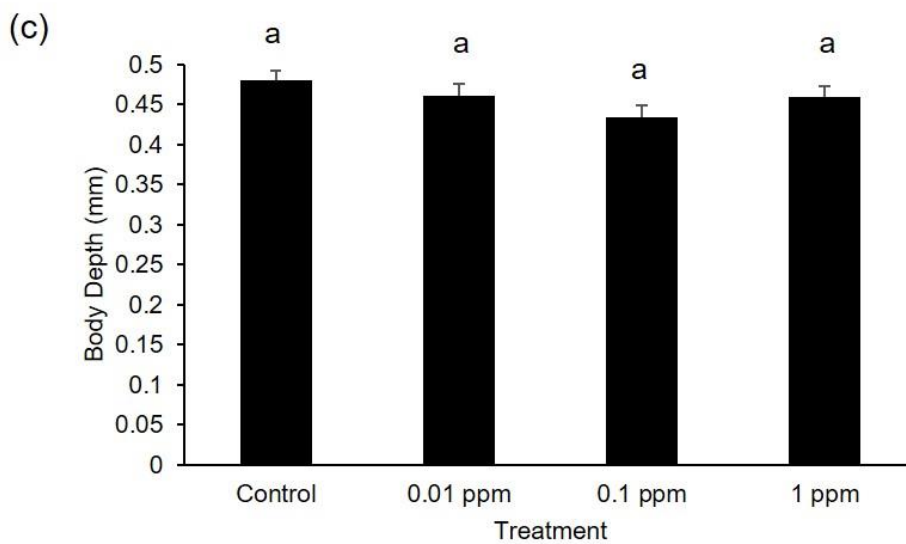
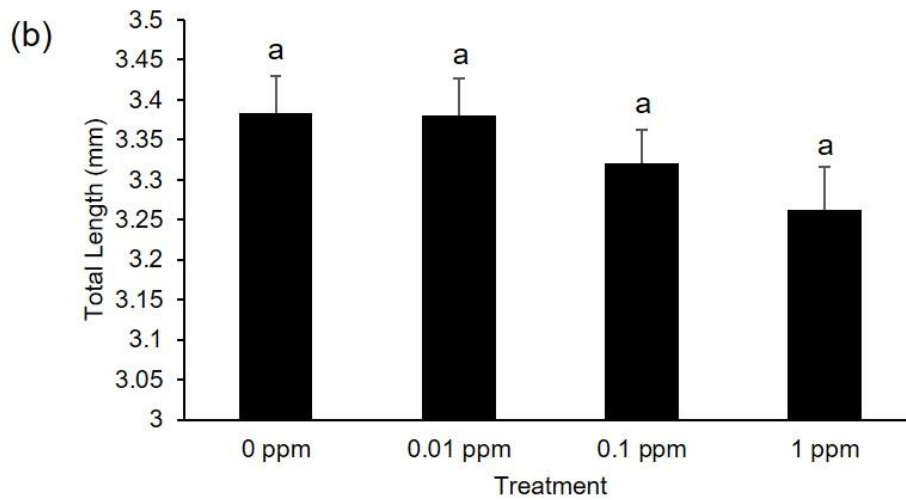
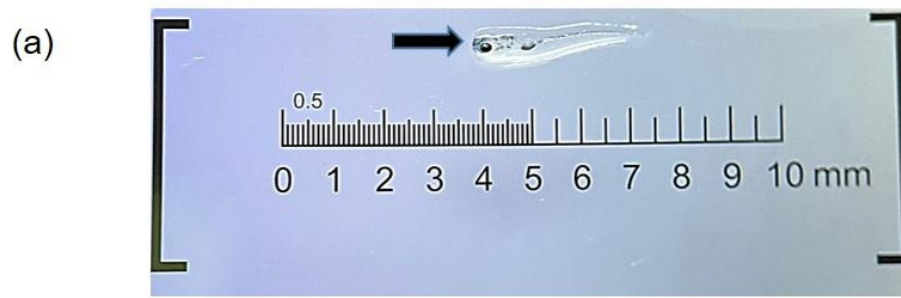


Fig. 7 Body biometry of Lemon Fin Barb Hybrid larvae. (a) Representative image of the larvae (arrow) sampled from the control group at 7 hr post-hatching, observed under the stereomicroscope A bar graph showing the (a) total length and the (c) width of the larvae exposed to different concentrations of endosulfan (either at 0.01, 0.1 and 1 ppm). The control group was placed in the environment absent of endosulfan. Data are mean \pm S.E.M.

5.0 DISCUSSION

One of the effects of ES is it may lead to reproductive dysfunction either in the male or female fish (Chakravorty et al. 1992; Rajakumar et al. 2012; Chakrabarty et al. 2012). ES can disturb the immunity and body defense of aquatic animal due to the inhibition of vitellogenesis (Chakravorty et al., 1992). Vitellogenin, a female-specific reproductive protein, plays an important role in providing the nutrients for developing embryo and involve in the immune defence against microorganisms like bacteria and viruses (Zhang et al., 2015). Slow ovarian activity, abnormal ovarian wall thickness, disruption of oogenesis are other effects of ES on female reproduction (Pandey, 1988). In male fish, ES was found to impair testis development, particularly disrupting the spermatogenesis, as well as reduce plasma testosterone, sperm motility and sperm count (Choudhary and Joshi, 2003; Rajakumar et al. 2012; Balasubramani and Pandian 2008). These evidences supported our findings, where the fertilization and hatching rate was reduced significantly in the ES exposed group (Section 4.1). We hypothesized that ES might impair the structure and functions of LFBH gametes, lead to reduce the conception rate between the sperms and the eggs. We propose further studies need to be conducted to attest our hypothesis.

In this study, we expected that ES exposure would have a negative effect on the physical growth of the LFBH larvae (Section 4.4). However, our evaluation on the body biometry of the larvae at 76 h post-hatching revealed no significant differences. This is in contrast with the studies by Balasubramani and Pandian (2008) in Zebra fish, and Sharmila and Abhik (2013) in snakehead fish, where suppression of growth was observed in the two species. ES was found to accumulate in the muscle of Zebra fish

and resulted in impaired swimming. This further impaired the foraging efficiency and reduced the digestion and absorption of the foods (Balasubramani and Pandian, 2008). The same effects of ES were not translated in our study as it was only assessed for a short duration (3 days). In other studies, where significant changes of body biometry were observed, the experiments were conducted at a longer duration (>21 days) (Sharmila and Abhik, 2013).

Finally, ES was found to significantly reduced the survival rate of LFBH larvae over time (Section 4.3). ES are known to possess disruptive effect to the osmoregulatory function of aquatic organisms (Saravana Bhavan, 2000; Altinok and Capkin, 2007; Bernabo et al. 2010). For example, a study by Bhavan, (2000) demonstrated significant structural changes in the gill lamellae of prawns exposed to ES. Another study in a rainbow trout reported the presence of lamellar oedema, separation of lamellae epithelium, lamella fusion and swelling of the epithelial cells of the gills (Altinok and Capkin, 2007). The gills are an essential organ, responsible for gaseous exchange, osmosregulation, mineral ion and ph homeostasis, excretion of nitrogenous waste and hormone production (Saravana Bhavan, 2000; Herrero et al. 2018). In a nutshell, impairment of the gill's function can cause the fish to unable to adapt to the changes in the environmental salinity, and finally leading to their death (Su et al. 2002).

In this present study, we also monitored the embryonic development of LFBH (Section 4.2). Our observation was consistent with the report by Romano et al. (2018) whom also looking into the embryonic development of the same hybrid in a normal environmental setting. The LFBH reached complete organogenesis at ~14h post-fertilization. This appear to be faster than the rosy barb which may take ~18h to reach

the same stages (Bhattacharya et al. 2012), Since LFBH carried half barb genetics, our findings suggest that hybrid may tend to have a faster embryonic development rate than its parent stock. This is further supported by the fact that *Barbonymus goniotus* took ~15h to reach complete organogenesis and ~16 for hatching (Myint and Soe, 2020).

6.0 CONCLUSION

ES exposures reduce the fertilization, hatching and survival rate of LFBH eggs and hatchlings. The data presented in this study provide insights into the effects of pesticides residues on the growth and production of native freshwater fish.

7.0 RECOMMENDATION

Among the limitations in this study is the short duration of the experiment. Although we expected that ES would possess a significant impact on the body biometry of the LFBH larvae, the differences of the total length and body depth was not significant at 72 hr. Therefore, longer study period, > 21 days is recommended for future studies such as implied in the studies by Sharmila and Abhik (2013) in order to see a clear view body biometry difference.

This present study focuses on the effects of ES on the early life stages of LFBH development. Future studies should look into the long-term effects of ES such as on the embryonic development, reproduction, transgenerational development and also the residues in fish tissue. This model can be used as a reference to examine the effects of pesticide residues on other Malaysian native fish, such as Patin, Kelah, Sebarau and Jelawat. Ultimately, the data will provide valuable information not only on the impact

of agricultural activities on the biology of native fish and the management of freshwater fish farming, but also policies and regulations of pesticide use in Malaysia.



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