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Original Article

Salinity effect on fry development of hybrid Malaysian mahseer (*Tor tambroides* ♂ × *Barbonymus gonionotus* ♀)♣

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Abstract

The purpose of this study was to analyse the influence of water salinity on fry development of hybrid Malaysian mahseer under controlled conditions. The physical changes and the tolerance limits in stress condition due to salinity exposure were determined in a recent study. The experiment was conducted in the wet laboratory, Department of Aquaculture. Ovatide hormone (0.4 ml/kg) was used to induce ovulation of female *Barbonymus gonionotus*, while the sperms were collected from the male mahseer. The recent study was done to investigate the effects of salinity on hybrid fish in terms of survival, growth, histological changes, blood content and hepatosomatic index (HSI) under laboratory conditions. This study exposed the fish to different concentrations of salinity (0, 3, 6 and 9 ppt) for 3 months. All treatments were in triplicate, labelled and arranged in a randomized design. Fry were fed until satiation twice a day and 30% of water was changed daily. The highest growth rate and survival rate (84%±7.78%) of fry were obtained in 3 ppt water salinity, while the lowest growth performance and survival rate (67%±13.45%) occurred at 0 ppt. The highest body gains and lengths of fry were observed in 9 ppt water salinity. However, significant histological alterations were observed in 9 ppt water salinity which caused major anomalies such as structural alterations in portal vein, vacuolation, necrotic hepatocytes, aggregation of blood cells and melanomacrophages. New strains of hybrid Malaysian mahseer were successfully bred. Optimal condition for fry Malaysian mahseer hybrid was 3 ppt water salinity. The results show that excessive salinity in the aquatic conditions affected negatively the health of the hybrid fish.

Keywords: Malaysian mahseer hybrid, water salinity, fry development, hepatosomatic index, liver histology

1. Introduction

Nowadays, the supply of *Tor tambroides* is quite constrained because it relies upon catches from the wild. This species has become highly demanded and popular as food and for fishing sport in Malaysia. However, this species breeds only in a certain season, has slow growth rate, and has become endangered, hence making it impossible to meet the recent market demand. Reproduction in captivity is normally impeded and therefore needs to be induced. Recently, hybrid production has become a very important way to resolve this problem for the endangered fish. Hybridization usually aims to provide positive traits to improve the performance of fish, such as improved disease resistance, good flesh quality, sex ratio control, and significantly improved fish tolerance of extreme environments (Nguenga *et al.*, 2000). The motivation of hybridization is to develop a hybrid that has characteristics superior to both parents (hybrid vigor) or is economically more profitable than its parent species. Hybrid fish between Malaysian mahseer (*Tor tambroides*) and silver barb

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(*Barbonymus gonionotus*) were introduced in this study to overcome this problem and to maintain mahseer traits while producing a good quality of hybrid fish. In prior research, no similar study has been done due to the lack of records on the breeding history of this new hybrid.

The performance of the new hybrid was tested and water salinity was selected as our experimental parameter. It is commonly known that high tolerance of water salinity reduces the chances of disease occurrence in freshwater fishes. Besides, the parameters such as embryo survival (egg incubation), larvae survival (rearing time), abnormal mortality percentage during the early development, and the growth rate of fry are affected by water salinity. Water salinity affects the early stages, particularly of larvae, as the larvae are more vulnerable than adults to external chemical stressor (Varsamos, Nebel & Charmantier, 2005) and have negative impacts on growth (Boeuf & Payan, 2001; Engström-Öst *et al.*, 2005). Survival and growth rates are keys for successful early larval rearing (Appelbaum & Kamler, 2000). However, the best conditions of water salinity for the growth and survival of these hybrid larvae remain unknown. Sufficient fish stock production depends on good conditions of egg incubation and fry growth (Kujawa *et al.*, 2017). Furthermore, water salinity is restricted to the aquatic environment and therefore the water salinity level impacts most aquatic organisms in their egg fertilization, early embryogenesis and fry growth (Boeuf & Payan, 2001). In addition, water salinity may simply be beneficial, by controlling and reducing the circumstances of a disease (Resley *et al.*, 2006). Previous studies have demonstrated fingerlings and post larval stages handling in a low salinity as a preventive measure against pathogen contamination, as infectious illnesses generally attack an early larval culture (Rigos & Smith, 2015), but the conditions are not favourable anymore when the salinity exceeds 6 ppt due to increased larval mortality (Fashina & Busari, 2003).

Detailed experimental studies were performed recently addressing growth development, histology and hepatosomatic index (HSI) to discover the effects of water salinity exposure to the early development of this hybrid. Growth is a physiological function influenced by salinity in the fish culture. Both parents of this hybrid (*Tor tambroides* and *Barbonymus gonionotus*) are freshwater fish. Some freshwater species have higher growth and survival rates of fry culture at low water salinities than in the freshwater (Britz & Hecht, 1989). Histological study helps in detecting any subtle conditions or morphological changes in liver tissue caused by different water salinity exposure, which could not be recognized by a gross examination without tissue structure observation.

As this is a new hybrid in Malaysia, further study should be done to improve and to increase production while preventing *Tor* traits from extinction. Therefore, the aim of this study was to analyse the effects of water salinity on the fry development of Malaysian mahseer hybrid.

2. Materials and Methods

2.1 Experimental design

The experiments were conducted in the wet laboratory in the Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor. The present study was carried out by induced breeding on 23rd May 2017 using males of *Tor tambroides* and females of *Barbodes gonionotus* as the brood fishes. Ovatide hormone was used to induce breeding with the dosage of 0.4ml/kg to a silver barb female. The fish were exposed to different salinities of 0, 3, 6, 9 ppt during the experiment. The embryonic development was observed 24 h starting from fertilization until hatched. The fish were randomly distributed among 12 aquariums (40cm × 40cm × 45cm) and each aquarium contained 15 larvae with gentle aeration. Salinities were recorded with a refractometer weekly to maintain the salinity level. All treatments were in triplicate, labelled and arranged in a randomized design. The fish were fed with commercial powder feed, FS Feed® (41% protein) until satiation twice a day, and 30% water was changed once every alternate day.

2.2 Growth development of cultured fish

The body weight and the total length of fish were measured by using electronic balance and Vernier caliper, respectively, once in 2 weeks for each treatment. The fish were monitored daily until 3 months of the experiment. The fish were anesthetised using AQUI-S to avoid stress during handling. Length-weight relationships of the fish for the initial and the final weeks were assessed to determine whether the somatic growth was isometric or allometric.

2.3 Calculation of hepatosomatic index

The body mass in each treatment, including the control fish, was recorded. Then, the liver representing each treatment was gently dissected out and weighed for Hepatosomatic index (HSI) calculation using the following formula:

$$HSI = \left(\frac{liver\ weight\ (g)}{total\ body\ weight(g)}\right) \times 100$$

2.4 Liver histological analysis

Liver tissues from the selected fish representing each treatment were collected after the experiment ended and weighed before immediately fixing in 5% formalin for further analysis. The preserved tissues were then subjected to standard histological procedures started with dehydration in an alcohol series followed by xylene, and embedding in paraffin blocks. The samples were sectioned to 7µm thick slices using an ultra-microtome (Thermo scientific HM340E) and stained with haematoxylin and eosin. Slides were mounted with DPX for stain preservation. The slides were observed and captured using a microscope (Leica DM750) supported by Dino-eyes.

2.5 Blood sample collection

Several fish were randomly selected from each treatment (0, 3, 6, or 9 ppt) and placed into preparation tanks (50L) with aeration provided. All the fish were anaesthetized with AQUI-S New Zealand Ltd (540g/L isoeugenol). The fish were fasted for 24 hours before blood drawing. The blood from each treatment was collected by caudal venepuncture using 2ml syringes. Blood samples were collected into blood tubes with no anticoagulant. The whole blood was left for 30 minutes in order to obtain plasma. All plasma were separated from the blood using centrifugation at 5000rpm for 10–15 minutes and the samples were kept at -20°C for biochemical indices analyses at laboratory of Pathology and Microbiology of Faculty of Veterinary Medicine, UPM.

2.6 Statistical Analysis

Each treatment was replicated for three times and all the data are described as mean with standard error (SE) to find significant differences between the treatments. One-way analysis of variance (ANOVA) was conducted for statistical analysis by using R Software. *Post hoc* analysis was done by Duncan's test to compare the treatment means.

3. Results and Discussion

3.1 Fry performance

Malaysian mahseer hybrid was able to absorb the egg yolk and had 100% survival for up to 4 days after hatching in control and in all treatments with salinity. The growth performance in all treatments increased gradually until week 6, with the highest total length and weight recorded for 9 ppt water salinity at 62.39mm±1.67 and 2.88g±0.21 (n=40), while the lowest total length and weight were observed for 0 ppt at 56.85mm±1.99 and 2.58g±0.20 (n=40). There were no significant differences in the final body lengths and weights among the treatments (Table 1). However, salinity tolerance also affected the survival of hybrid fish. Higher salinity caused a higher mortality. The survival rate was high in all experimental groups (0, 3, 6, 9 ppt) throughout the first set of measurements with 100% survival. Fish started to die at week 8 with 33% and 31% mortalities in 0 ppt and 9 ppt, respectively. At the end of the experiment, the highest final survival rates were observed for 3 ppt and 6 ppt water salinities, reaching 84% and 71% respective rates. The results might indicate that new hybrid fish were able to survive and regulate their osmotic pressure in a low salinity water. Similar studies have tested 3 ppt to 9 ppt water salinity effects on various fish fry such as rainbow trout Oncorhynchus mykiss, Gulf sturgeon Acipenser oxyrinchus desotoi, and striped bass Morone saxatilis (Altinok & Grizzle, 2001). In contrast, a drastic reduction in survival was observed for the highest tested salinity (9 ppt) and for the control group (0 ppt) after 40 days of experiment. The mortality might be because of high energy consumption (exhaustion) due to the reduction of mucous cells within the gills, which utilized glycogen within liver and intestine. Besides, the fish tended to use more energy on osmotic and ionic regulation in higher salinities, which caused energy depletion, hence limiting development and growth. Thus, a near optimal salinity is essential due to energetic demands of osmoregulation, particularly during the early nursery stage, to avoid severe stress (Romano *et al.*, 2017). Since growth was compromised, water salinity of 3 ppt showed a lower metabolic demand for osmoregulation. Thus, this condition will improve growth development if the larvae are exposed for a prolonged period. The effects of water salinity on fry survival are illustrated in Figure 1.

The measurement for all treatments on the last day found that the highest mean body weight attained from fry culture was for 9 ppt water salinity (Figure 2). However, no significant result was observed on the growth development between groups.

In addition, the highest average total body length was achieved in 3 ppt and 9 ppt water salinities. The results were similar to body weight gains but the differences between particular groups were less distinct. Fry growing in 6 ppt water salinity gave on average 7.08 mm shorter length at the end of the rearing period than 3 ppt salinity (Figure 3).

The daily mortality rate of hybrid was closely correlated with the water salinity. The smallest number of dead fries occurred in 3 ppt salinity. During the last week of experiment, the number of dead individuals in this treatment exceeded 30% of total stock. The dead individuals showed signs in the body anatomy and behaviour, such as discoloration, weakness, and refusal to feed, which were most probably responsible for their death.

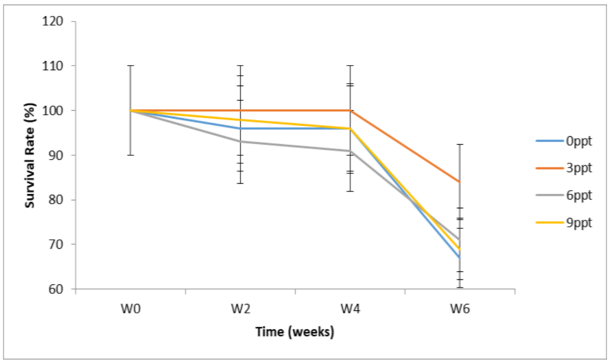


Figure 1. Survival of Malaysian mahseer hybrid fry reared in various salinities *** W0: week 0; W2: week 2; W4: week 4; W6: week 6

Table 1. Result of Malaysian mahseer hybrid fry reared in water with various salinities. Mean ±SE; n=40

Parameter	Initial	Salinity (ppt)		
	0	3	6	9
Initial mean body weight (g)	0.43±0.03[a]	0.41±0.03[a]	0.40±0.04[a]	0.50±0.04[a]
Final mean body weight (g)	2.58±0.20[a]	2.73±0.24[a]	2.61±0.23[a]	2.88±0.21[a]
Initial mean body length (mm)	31.31±0.86[ab]	30.31±0.94[ab]	29.97±0.91[a]	32.79±1.05[a]
Final mean body length (mm)	56.85±1.99[a]	60.55±2.06[a]	59.03±1.74[a]	62.39±1.67[a]
Initial stock (indiv.)	45	45	45	45
Final stock (indiv.)	30	38	32	31
Final Survival (%)	67	84	71	69

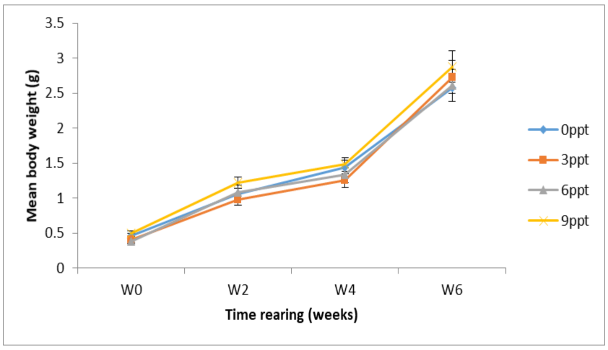


Figure 2. The mean (±SE) weight growth of Malaysian mahseer hybrid in various salinities

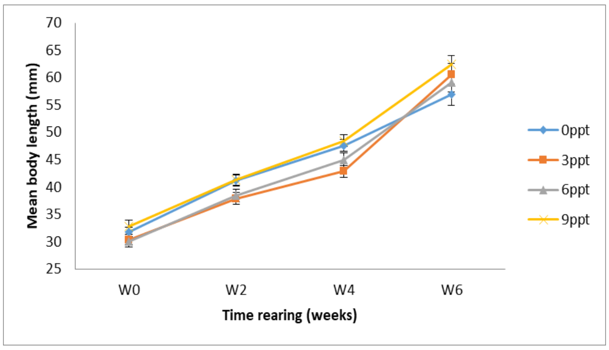
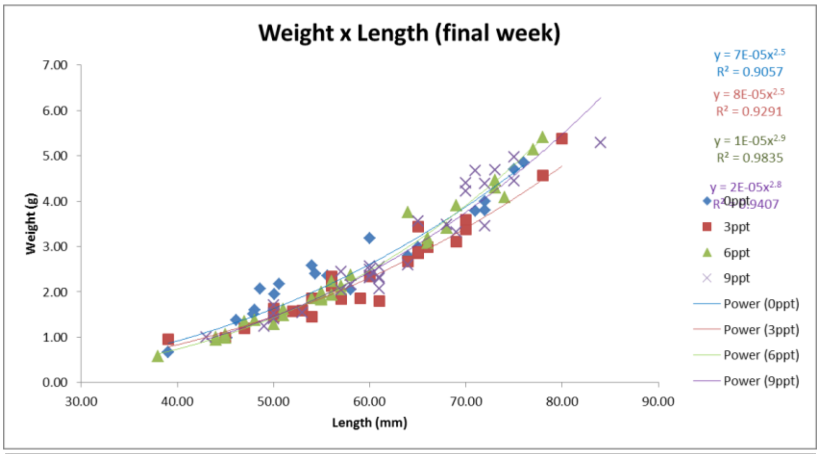


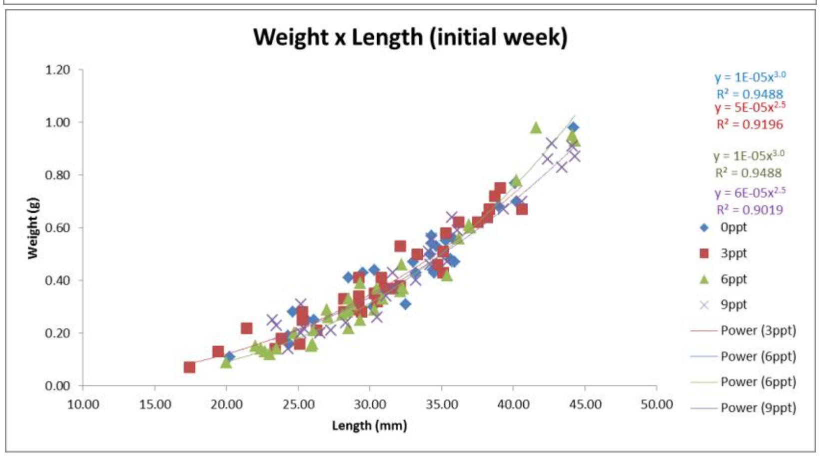
Figure 3. The mean (±SE) length growth of Malaysian mahseer hybrid in various salinities

Body weight and length of each fry were measured initially and in the final week to facilitate direct comparison between all actual treatments and the control group (Figure 4a, b). As expected, the body weight was inversely related to body length in all treatments groups. A scatter plot shows similar patterns for the initial and the final week.

In the results, the b values of the control, 3 ppt, 6 ppt and 9 ppt water treatments in the initial week were 3.0, 2.5, 3.0 and 2.5, respectively, which means that 0 ppt and 6 ppt had values close to 3 (isometric growth pattern) while 3 ppt and 9 ppt had below 3 indicating negative allometric growth. Negative allometric growth implies the fish become more slender they grow longer. The coefficient of determination (R^2) for the control and all treatments during the initial week ranged from 0.9019 to 0.9488. Compared to the final week, the b values of the control, 3 ppt, 6 ppt and 9 ppt water treatments (2.5, 2.5, 2.9 and 2.8, respectively) were all below than 3, indicating that all had negative allometric growth. The determination coefficient (R^2) for the control and all treatments for the final week ranged from 0.9057 to



(a)



(b)

Figure 4. Body length (mm) in relation to body weight (g) of hybrid fishes in (a) the control and (b) actual treatment groups for the initial and the final weeks. Fish were fed to satiation two times daily on a commercial diet. Each data point represents a subsample of 40 fish.

0.9835. The Linear Allometric Model analysis of hybrid Malaysian mahseer fry showed that there was variation in b values for the initial week and the final week samplings, where the value for the initial week tended to 3 (isometric growth pattern) for the control and 6 ppt water salinity while it tended to be below 3 (negative allometric growth pattern) for 3 ppt and 9 ppt water salinities. This variation of growth pattern might happen because of competition for external food to increase survival during the early weeks when the fry completed their morpho-functional systems. According to Bailey & Houde (1989), famine and cannibalism are the main aspects of fry mortality during the early stages of life. Besides, feed and locomotor systems also need to evolve simultaneously and in mutual equilibrium (Osse *et al.*, 1997). The b values for the final week were seen to be below 3 in all treatments, for negative allometric growth pattern. This happened probably because of increasing body length along the weeks due to genetic modification, so that the hybrid fishes might resemble Malaysian mahseer in an elongated body characteristic. Genetic and environmental factors influence the b value (Nash *et al.*, 2006). In addition, the variation in b values might also be influenced by changes in food availability, gonad maturation, age, gonad production, season, habitat, feed, stomach fullness, salinity, and temperature (Moradinasab *et al.*, 2012).

3.2 Hepatosomatic index (HSI)

Hepatosomatic index provides vital information about the condition of liver and body and also about the impacts of water salinity or pollution. As the weight of body increased, the weight of liver also increased. HSI also serves as an indicator of state of energy reserve in a fish. HSI values were found to be little below the normal with higher salinity exposure. More than 30% mortality was recorded during the experiment in 0 ppt and 9 ppt groups surpassing the other groups (3 ppt and 6 ppt). HSI value for the control group was 5.66±0.49, while HSI for the other treatments at 3 ppt, 6 ppt and 9 ppt were 6.02 ± 2.23, 3.48 ± 1.80 and 7.07 ± 2.93, respectively. The higher salinity exposure affected physiological activity of the fish liver distinguishing it from 3 ppt water salinity and the control without salinity. The decrease in HSI might be due to atrophy or necrosis of hepatocytes as described by Busacker *et al.* (1990). Fish usually have smaller liver with less energy reserve in a poor environment.

3.3 Histological observations

Figures 5a-b and 6a-b show that 0 ppt (the control group) and 3ppt demonstrated normal histological architectures in the liver tissue, indicating the fry could grow and survive in those salinities. However, morphological alterations of liver tissue such as congested portal vein started to appear in 6 ppt treatment group (Figure 7a-b). Besides, congested portal vein, degradation in pancreatic tissue, necrotic hepatocytes, vacuole formation and initialized blood cell aggregation were more strongly observed in the 9 ppt treatment group (Figure 8a-c). Similar to the present study, salinity affects growth and survival of the sichel *Pelecus cultratus* (L.) larvae, and the lowest growth and survival of sichel larvae occurred in 9 ppt water salinity.

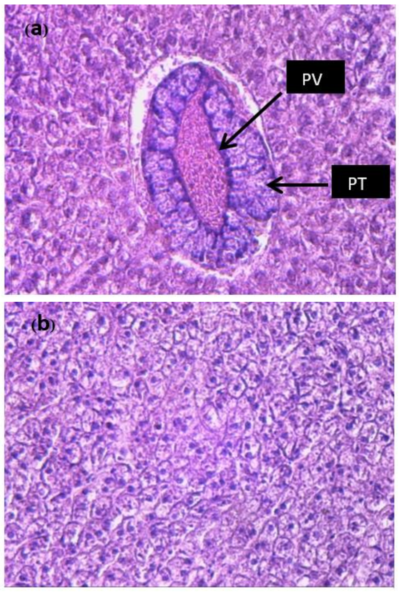


Figure 5. 0ppt- Histological sections from liver tissue of Malaysian mahseer hybrid. (a) Photomicrograph showing normal architecture of liver section in areas surrounding the portal vein (PV) and exocrine pancreatic tissue (PT) (10×). (b) Normal fish liver tissue (10×)

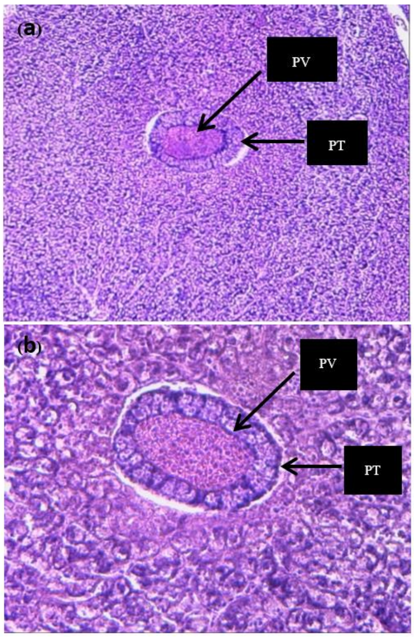


Figure 6. 3ppt- Histological sections from liver tissue of Malaysian mahseer hybrid. Photomicrograph showing normal architecture liver section in areas surrounding the portal vein (PV) and exocrine pancreatic tissue (PT) (a) 10×, (b) 20×

Histological lesions in the liver tissues were scored based on their severity and are summarized in Table 2. Normal histological architecture was found and pathological abnormalities were not seen in the liver tissues from the control fish and 3 ppt treatment groups. High levels of blood

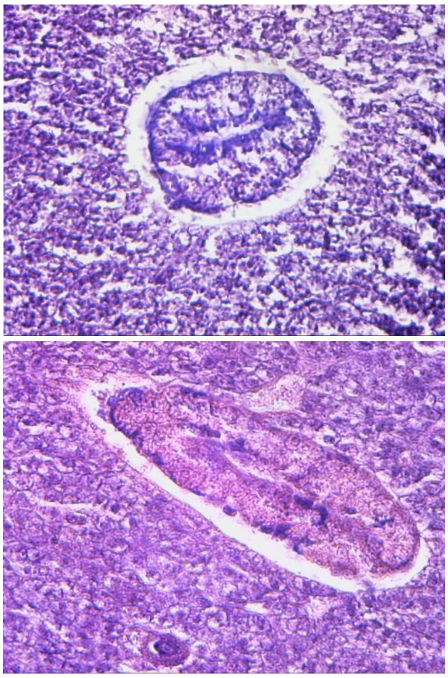


Figure 7. 6ppt- Histological sections from liver tissue of Malaysian mahseer hybrid. Photomicrograph shows degradation of fish liver section in areas surrounding the congested portal vein (PV) and pancreatic tissue (PT) (a) 10×, (b) 10×

Table 2. Summary of the liver histological changes in the fish exposed to various salinity levels

Histological	Control (0 ppt)	Salinity concentration		
		3ppt	6ppt	9ppt
Congestion of blood vessels	-	-	+	+++
Aggregation of blood cells	-	-	+	++
Degradation of liver tissues	-	-	+	++
Necrosis	-	+	+	+++
Foci of hepatitis-like injury	-	-	+	++
Foci of melanomacrophages	-	-	+	++
Mean	-	-	+	++

-: None (zero %), +: mild, ++: moderate, +++: severe

vessel congestion and hepatic tissue degeneration were more intensive at higher salinities, most so in the 9 ppt treatment group. Severe necrosis was also observed in the liver tissues of fry exposed to 9 ppt. Increased pigmentation (melano macrophage) acts as an indicator of stressful conditions in aquatic environment (Agius & Robert, 2003) and also affects the recycling of endogenous substances from damaged cells (Passantino *et al.*, 2014). Therefore, lower salinities (e.g., 1–3 ppt) can be used in hatcheries to minimize the disease-related mortality in freshwater fish (Altinok & Grizzle, 2001). Freshwater fish are capable of tolerating salinity changes depending on their ability to retain body osmoregulation by releasing copiously urine as well as by active uptake of ions through gills (Sahoo *et al.*, 2003; Kültz, 2015). Furthermore, salinity intrusion in freshwater aquaculture areas leads to salinity stress and some organisms may die due to their inability to overcome the stress. Therefore, this ability and

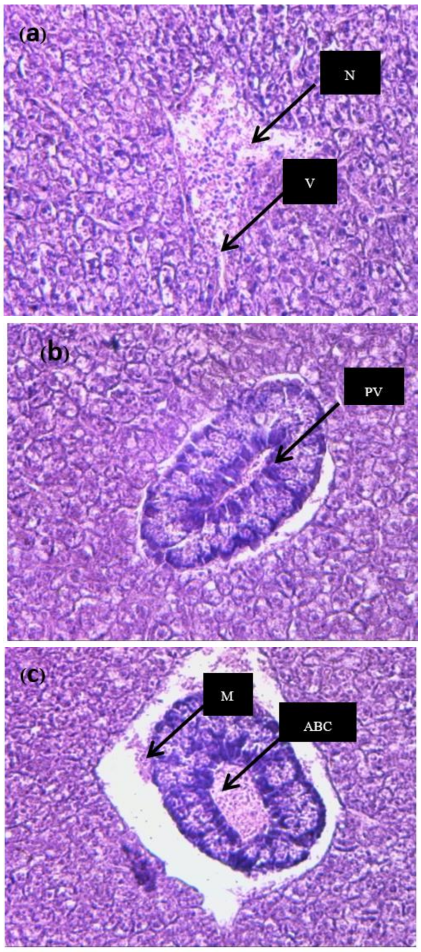


Figure 8. 9ppt- Histological sections from liver tissue of Malaysian mahseer hybrid. (a) Necrosis (N) and vacuolation (V), (b) congested portal vein (PV) and pancreatic tissue (PT) (10×), (c) melanomacrophage (M), aggregation of blood cells (ABC) and degradation in the pancreatic tissue (PT) of liver tissue (10×)

tolerance of salt are crucial in designing some treatments for disease and stress reduction.

3.4 Blood biochemical indices

The plasma osmolality results showed a direct relationship with salinity increase. Higher survival rates can be achieved with the acclimatization of this species to different water salinities (Mattioli *et al.*, 2017). The blood chemical parameters for sodium (Na), potassium (K), chloride (Cl), total protein, glucose (GLUC), and cholesterol (CHOL) in the hybrid are shown in Table 3. Continuous adaptation to the concentrations of Na+, K+ and Cl- takes place in order to maintain the homeostasis in balance. To compensate with a sufficient salt level, special cells in the gills (chloride cells) take up ions from the water, which are then directly transported into the blood. The blood factor results of hybrid for Na^+, K^+ and Cl^- ions increased with water salinity. However, they slightly decreased in 9 ppt. The petite increment in ion concentrations did not exert a significant

Table 3. Biochemical properties of blood in Malaysian mahseer hybrid at the end of experiments

Salinity (ppt)	Osmoregulation factors			Stress factors		
	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L	Gluc mmol/L	Chol mmol/L	Total protein g/L
0	140	1.0	102	4.2	6.7	28.4
3	165	1.5	111	3.1	9.1	36.8
6	177	2.0	126	2.9	9.8	38.3
9	200	3.0	150	3.1	9.2	35.2

effect to the osmotic balance (extreme hypertonic) and allowed salinity stress. This new hybrid appears to be a euryhaline fish that is tolerant to water salinity. Nile tilapia *Oreochromis niloticus* was reported to tolerate up to 20 ppt in spite of being a freshwater fish (El-Sayed, 2006; Jun *et al.*, 2012). The lowest cholesterol and total protein were in the control group, while the highest were for 6 ppt and 9 ppt treatments. Consequently, the hybrid fish exhibited minor stress during the treatment period to maintain the transport of oxygen to fulfil the biological demand by the tissues. However, the hybrid was able to maintain stable blood osmolality. Glucose was utilized as an indicator of overall metabolic changes in the animal body (Mattioli *et al.*, 2017). In this study, glucose level seemed to be decreasing with salinity. The reduction of glucose levels might induce muscular depletion similar to diminution of dietary intake, caused by stress. The results indicate that exposure to lower salinities in early stages of hybrid offer positive results in growth, indicating a less stressful environment. High level of cortisol from long-term stress might be the mechanism via which stress could increase cholesterol.

4. Conclusions

Based on observations the present experimental study corroborated the statement that optimal rearing conditions for Malaysian mahseer hybrid have between 3 ppt and 6 ppt salinity. However, the Malaysian mahseer hybrid can survive and grow in 9 ppt rearing conditions. We observed that the fish showed great adaptation to all salinity treatments and high survival rates during the study period.

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