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 the 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 readily male mahseer. Recent study was done to investigate the effect 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 triplicated, 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 water salinity 3 ppt, while the lowest growth performance and survival rate (67%±13.45%) occurred in water 0 ppt. The highest growth rate and survival rate (84%) of fry were obtained in water salinity 3 ppt, while the lowest growth performance and survival rate (67%) occurred in 0 ppt. The highest body gains and lengths of fry were observed in water salinity 9 ppt. However, significant histological alterations were observed in water salinity 9 ppt which caused major histological 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 obtained in water salinity 3 ppt. Results have shown that high salinity in the aquatic condition affected the health condition 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 restrained because it relies upon the availability from the wild. This species becomes highly demanding and popular as food and sport fish in Malaysia. However, this species which breeds only in certain season has slow growth rate and becomes endangered, hence making it impossible to meet recent market demand. Reproduction in captivity is normally impeded and therefore need to be induced. Recently, hybrid production becomes a very important way especially to resolve this endangered fish’s problem. Hybridization usually aims positive traits to improve performance of fish such as increases diseases resistance, good flesh quality, sex control ratio and significantly improve fish tolerance in extreme environment (Nguenga et al., 2000). The desire of hybridization is to develop a hybrid that has characteristic superior to both parents (hybrid vigor) or economically profitable than its parent species. Hybrid fish between Malaysian mahseer (*Tor tambroides*) and silver barb (*Barbonymus gonionotus*) were introduced in this study to overcome this problem and maintain mahseer traits while producing a good quality of hybrid fish. From previous research, there is no study yet had been done due to lack of records on the breeding history of this new hybrid. The performance of new hybrid was tested and water salinity was selected as our experimental parameter. It is commonly known that, high tolerance towards water salinity reduces the chances of disease occurrence in freshwater fishes. Besides, the parameters such as embryo survival (eggs incubation), larvae survival (rearing time), abnormal percentage occurring during the early development and the growth rate of fry are affected by water salinity. Water salinity affects the early stage of larvae particularly,
as larvae are more vulnerable than adult in terms of external body, chemical stressor (Varsamos, Nebel & Charmantier, 2005) and growth (Boeuf & Payan, 2001; Engström-Öst et al., 2005). Survival and growth rates are keys for the successful of early larval rearing (Appelbaum & Kamler, 2000). However, the best condition of water salinity for the growth and the survival of these hybrid larvae is still unknown. The sufficient fish stock production is depending on a good condition of eggs incubation and fry growth (Kujawa et al., 2017). Furthermore, water salinity is restricted to the aquatic environment and therefore the water salinity level gives an impact in most aquatic organisms such as egg fertilization, early embryogenesis and fry growth (Boeuf & Payan, 2001). In addition, water salinity may simply be beneficial, such as controlling and reducing the circumstance of disease (Resley et al., 2006). The previous studies have demonstrated fingerlings and post larvae are handled in low salinity as the prevention indicator towards the pathogenic contamination in view that infectious illnesses generally attack early larval culture (Rigos & Smith, 2015), but the conditions are not favourable anymore when the salinity reach above 6 ppt due to increase in larval mortality (Fashina & Busari, 2003).

Detailed studies were performed in recent experiment in terms of growth development, histology and hepatosomatic index (HSI) to discover the effect of water salinity exposure to the early development of this hybrid. Growth is a physiological function influenced by salinity concentration in 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 in comparison to 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 that cannot be recognized on gross examination with 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 trait from extinction. Therefore, the aim of this study was to analyse the effect 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 technique on 23rd May 2017 using males Tor tambroides and females Barbodes gonionotus as the brood fishes. Ovatide hormone was used for induced breeding with the dosage of 0.4ml/kg for 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 reading
were recorded with refractometer weekly to maintain the salinity level. All treatments were triplicated, 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 calliper, 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 conducted to determine whether the somatic growth was isometric or allometric.

2.3 Calculation of hepatosomatic index

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

$$\text{HSI} = \left( \frac{\text{Liver mass} \text{ (g)}}{\text{Body mass} \text{ (g)}} \right) \times 100$$
2.4 Liver histological analysis

Liver tissues from the selected fish of each treatment were collected after the experiment ended and weighed before immediately fixed in 5% formalin for further analysis. The preserved tissues were then proceeded to standard histological procedure started with increasing alcohol dehydration series followed by xylene and embedded in paraffin blocks. The samples were sectioned at 7μm thick slices using an ultramicrotome (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, 9 ppt) and placed into preparation tanks (50L) with aeration provided. All fish were anaesthetized with AQUI-S New Zealand Ltd (540g/L isoeugenol). The fish were fasted for 24 hours before blood drawing process. The blood from each treatment was collected by caudal venepuncture using 2ml syringes. Blood samples were collected into blood tubes with no anticoagulation. 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 data were described as mean with standard error (SE) to find significant different between treatments. One-way analysis of variance (ANOVA) was conducted for statistical analysis by using R Software. Post hoc analysis was done by Duncan to compare the treatment means.

3. Results and Discussion

3.1 Fry performance

Malaysian mahseer hybrid was able to absorb their yolk and had 100% survival for up to 4 days after hatched in control and all treatments of salinity concentration. The growth performance in all treatments increased gradually by week until week 6 with the highest total length and weight were recorded in water salinity 9 ppt with 62.39mm±1.67 and 2.88g±0.21 (n=40) while the lowest total length and weight were observed in 0 ppt with 56.85mm±1.99 and 2.58g±0.20 (n=40). There were no significant differences in the final body lengths and weights among all the group treatments (Table 1). However, salinity tolerance also affected the survival of hybrid fish. Higher concentration of salinity caused higher mortality. The survival rate was high in all experimental groups (0, 3, 6, 9
throughout the first set of measurements with 100% survived. Fish group started to
die at week 8 with 33% and 31% mortality in both 0 ppt and 9 ppt, respectively. At the
end of the experiment, the highest final survival rates were observed in 3 ppt and 6 ppt
of water salinity, where they reached 84% and 71%, respectively. The results might
indicate that new hybrid fish were able to survive and regulate their osmotic pressure in
lower saline water. Similar studies on salinity effect were observed in 3 ppt to 9 ppt of
water salinity 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, huge reduction of survival was observed in the highest tested
salinity (9 ppt) and the control group (0 ppt) after 40 days of experiment. The mortality
occurred 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, fish tended to use more energy on osmotic and ionic regulation in higher
salinities, which caused energy depletion hence limiting development and growth. Thus,
optimum salinity is essential due to energetic demand for 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 lower metabolic demand for
osmoregulation. Thus, the condition will improve growth development if the larvae are
exposed for longer period. The effect of water salinity on the fry survival is illustrated in

*** W0: week 0; W2: week 2; W4: week 4; W6: week 6

Figure 1: Survival of Malaysian mahseer hybrid fry reared in different salinity
Table 1: Result of Malaysian mahseer hybrid fry reared in water with different salinity. Mean value ±SE; n=40.

The measurement for all treatments on the last day found that the highest mean body weight attained from fry culture in 9 ppt water salinity (Figure 1). However, no significant result was observed on the growth development between groups.

Figure 2: The mean (±SE) weight growth of Malaysian mahseer hybrid in different salinity

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

Figure 3: The mean (±SE) length growth of Malaysian mahseer hybrid in different salinity

The daily mortality rate of hybrid was closely correlated with the water salinity. The smallest number of dead fry occurred in water salinity 3 ppt. 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 the behaviour such as discolouration, weakness and refusal of feed, which were most probably responsible for their death.
Body weight and length of each fry were measured on the initial and the final week to facilitate direct comparison between fish for all treatments and the control group (Figure 4a, b). As expected, body weight was inversely related to body length in all treatments and the control group fish. Scatter plot graph below shows similar pattern for the initial and the final week.

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

Based on the result, the \( b \) value of the control, 3 ppt, 6 ppt and 9 ppt water treatments for the initial week were 3.0, 2.5, 3.0 and 2.5, respectively which means 0 ppt and 6 ppt had the values closed to 3 (isometric growth pattern) while 3 ppt and 9 ppt had lower than 3, that indicated negative allometric growth. Negative allometric growth implies the fish becomes more slender as it becomes longer. The determination coefficient \( R^2 \) for the control and all treatments during the initial week ranged from 0.9019 to 0.9488.

Compared to the final week, the \( b \) value of the control, 3 ppt, 6 ppt and 9 ppt water treatments (2.5, 2.5, 2.9 and 2.8, respectively) were below than 3 indicating that all have negative allometric growth. The determination coefficient \( R^2 \) for the control and all treatments for the final week ranged from 0.9057 to 0.9835. The Linear Allometric Model analysis of hybrid Malaysian mahseer fry showed that there was a variation of \( b \) values for the initial week and the final week samplings where the value for the initial week was 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 their survival during 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 than 3 in all treatments which tended to negative allometric growth pattern. This happened probably because of increasing body length along the weeks due to genetic modification which is hybrid fishes might resemble Malaysian mahseer elongated body characteristic. Genetic and environmental factors influenced the b value (Nash et al., 2006). In addition, the variation of b values also might be influenced by changes in food availability, gonad maturation, age, gonad production, seasons, habitat, feed, stomach fullness, salinity and temperature (Moradinasab et al., 2012).

Hepatosomatic index (HSI)

Hepatosomatic index provides vital information about the condition of liver and body and also about the impact of water salinity or pollution on it. As weight of body increased, weight of liver also increased. HSI also acted as indicator on status of energy reserve in fish. HSI values were found to be little below than normal values with the higher salinity exposure. More than 30% mortality was recorded during the experiment in
0 ppt and 9 ppt groups compared to other groups (3 ppt and 6 ppt). HSI value for the control group was recorded to be 5.66±0.49, while HSI value for other treatments of 3 ppt, 6 ppt and 9 ppt were found as 6.02 ± 2.23, 3.48 ± 1.80 and 7.07 ± 2.93, respectively.

The higher salinity exposure showed the physiological activity of the liver was affected compared to fish in 3 ppt water salinity and the control one. The decrease in the HSI values might be due to the result of atrophy or necrosis of hepatocytes as described by Busacker et al. (1990). Fish usually have smaller liver with less energy reserved in the poor environment.

**Histological observation**

Figure 5a-b and 6a-b show 0 ppt (the control group) and 3ppt demonstrated normal histological architecture in the liver tissue indicating the fry could grow and survive in that salinity range. However, morphological alteration on 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 observed in 9 ppt treatment group (Figure 8a-c). Similar to the present study, salinity affects the growth and the survival of the sichel *Pelecus cultratus* (L.) larvae where the lowest growth and survival of sichel larvae occurred in water salinity 9 ppt.

**Figure 5: 0ppt.** Histological sections for 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×)
Histological sections for 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 sections for 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) 20×

Histological sections for 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×)

Histological lesions of the liver tissues were scored based on their severity and are illustrated 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 level of blood vessels congestion and hepatic tissues degeneration was more intensive at higher salinity, mostly in 9 ppt treatment group. Severe necrosis was also observed in the liver tissues of 9 ppt exposed fry. Increased pigmentation (melanomacrophage) acts as an indicator of stressful condition 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 probable mortality in freshwater fish (Altinok & Grizzle, 2001). Freshwater fish are capable to tolerate salinities changes but it depends on their capability to retain body osmoregulation by releasing copious 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 tolerance of salt are crucial in designing some treatments for disease and stress reduction.

**Table 2**: Summary of the liver histological changes in the different salinity exposed fish.

**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 properties consisting sodium (Na), potassium (K), chloride (Cl), total protein, glucose (GLUC), and cholesterol (CHOL) in hybrid are shown in Table 3. Continuous adaptations with regards to the concentrations of Na+, K+ and Cl- takes place in order to maintain the homeostasis in balance. To compensate 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. Current blood factors results of hybrid including Na+, K+ and Cl- ions increased with salinity increased. However, it is slightly decreased in 9 ppt. The petite increment in ions concentration did not exert significant effect to the osmotic balance (extreme hypertonic) inside the blood that lead to salinity stress. This new hybrid appears to be euryhaline fish that is tolerant to water salinity. Nile tilapia *Oreochromis niloticus* was reported can 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 value was in the control group while the highest value was
in 6 ppt and 9 ppt. Consequently, hybrid fish exhibited minor stress during the treatment period to maintain the transport of oxygen to fulfill the biological demand of tissues. However, the hybrid showed they were capable to maintain stable blood osmolality. Glucose was utilized as most indicator for evaluation that induce entire metabolic changes of animal body (Mattioli et al., 2017). In this study, glucose level seemed to be decreasing with higher salinity. The reduction of glucose levels might influence muscular depletion due to diminution dietary intake caused by stress. The results indicated that the exposure of lower salinities to early stages of hybrid offer positive result in term of growth indicating a less stressful environment. High level of cortisol from long-term stress might be the mechanism behind how stress could increase cholesterol.

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

4. Conclusions

Based on that information, the present study strengthened the statement that optimal rearing condition for Malaysian mahseer hybrid is between 3 ppt to 6 ppt. However, Malaysian mahseer hybrid can survive and grow in 9 ppt rearing condition. We observed that the fish showed great adaptation to all salinity treatments and higher survival rate during the study period.

Acknowledgments
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References


Figure 1: Survival of Malaysian mahseer hybrid fry reared in different salinity

Figure 2: The mean (±SE) weight growth of Malaysian mahseer hybrid in different salinity

*** W0: week 0; W2: week 2; W4: week 4; W6: week 6
Figure 3. The mean (±SE) length growth of Malaysian mahseer hybrid in different salinity
Figure 4 (a, b): Body length (mm) in relation to body weight (g) of hybrid fishes in control and all treatments groups for initial and final weeks. Fishes fed to satiation two times/day on a commercial diet. Each data point represents a subsample of 40 fish.
Figure 5: 0ppt - Histological sections of control 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 X). (b) Normal fish liver tissue (10 X).

Figure 6: 3ppt - Histological sections of liver tissue of Malaysian mahseer hybrid. (a) Photomicrograph showing normal architecture liver section in areas surrounding the Portal vein (PV) and exocrine pancreatic tissue (PT) (10 X). (b) Showed normal fish liver tissue (10 X).

Figure 7: 6ppt - Histological sections of control liver tissue of Malaysian mahseer hybrid. (a) Photomicrograph showed degradation of fish liver section in areas surrounding the congested Portal vein (PV) and pancreatic tissue (PT) (10 X). (b) Showed congested Portal vein (PV) and pancreatic tissue (PT) (10 X).
Figure 8: Histological sections of control liver tissue of Malaysian mahseer hybrid. (a) Necrosis (N), Vacuolation (V) (b) Showed congested Portal vein (PV) and pancreatic tissue (PT) (10 X). (c) Showing melanomacrophage (M), Aggregation of Blood Cells (ABC) and degradation in the Pancreatic Tissue (PT)
**Table 1**: Result of rearing Malaysian mahseer hybrid fry in water with different salinity. Mean value ± SE; n=40.

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

**Table 2**: Summary of the liver histological changes in the different salinity exposed fishes.

<table>
<thead>
<tr>
<th>Histological</th>
<th>Control (0 ppt)</th>
<th>Salinity concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 ppt</td>
</tr>
<tr>
<td>Congestion of blood vessels</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Aggregation of blood cells</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Degradation of liver tissues</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Necrosis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Foci of hepatitis-like injury</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Foci of melanomacrophages</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mean</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>: None (zero %), <sup>b</sup>: mild, <sup>+</sup>: moderate, <sup>+++</sup>: severe.

**Table 3**: Biochemical properties of blood in Malaysia mahseer hybrid at the end of experiments.

<table>
<thead>
<tr>
<th>Salinity (ppt)</th>
<th>Osmoregulation factors</th>
<th>Stress factors</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Na&lt;sup&gt;+&lt;/sup&gt; (mmol/L)</td>
<td>K&lt;sup&gt;+&lt;/sup&gt; (mmol/L)</td>
</tr>
<tr>
<td>0</td>
<td>140</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
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<tr>
<td>---</td>
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</tr>
<tr>
<td>3</td>
<td>165</td>
<td>1.5</td>
</tr>
<tr>
<td>6</td>
<td>177</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>200</td>
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