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Improved performance of lemon fin barb hybrid (*Hypsibarbus wetmorei* ♂ *Barbodes gonionotus* ♀) at elevated salinities

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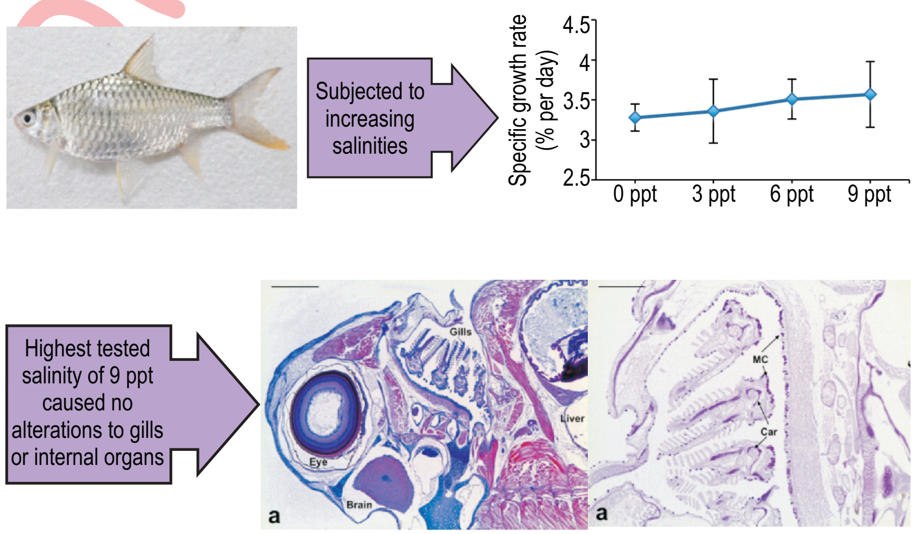
Abstract

Aim: Interest in lemon fin barb hybrid (LFBH) aquaculture has been increasing, but there is limited information regarding optimal nursery conditions.

Methodology: In this study, LFBH fry (initial mean weight = 1.3 ± 0.2 cm) were subjected to increasing salinities of 0, 3, 6 and 9 ppt. Each treatment was triplicated with 15 fish in each replicate. After 30 days, the survival, growth and whole-body histology was examined.

Results: No mortalities occurred over the study duration, but there was a significantly positive relationship ($p < 0.001$; $R^2 = 0.996$) between salinity and specific growth rates for weight. Salinity had no adverse effect on the gill or internal organ structure, as well as no effect to the prevalence of gill mucous cells. However, there appeared to be slightly more liver glycogen in fish subjected to 6 and 9 ppt. This study demonstrates the euryhalinity of LFBH over the more stenohaline characteristic of their parent, *Barbodes gonionotus.*

Interpretation: The euryhalinity of LFBH should provide more flexibility in their culture, such as polyculture with other brackish water species, as well as in areas experiencing increase salinization. Therefore, LFBH may provide greater food security in some areas.



Introduction

The early nursery culture of fish is a sensitive time for their development, and thus optimal environmental conditions should be provided in order to produce high quality seed for their subsequent grow out. Among the various water qualities, the optimal salinity range is highly species specific and deviations from this optimal range can reduce the overall condition, growth and survival of the fish (Bœuf and Payan, 2001). This is because salinity extremes can reduce energetic reserves, such as glycogen and/or lipids, by increasing energy demands for osmoregulation and/or disrupting normal metabolism (Febry and Lutz, 1987). Moreover, determining the performance of freshwater fish at increasing salinities may also have implications to disease management because slightly elevated salinities of 1 to 3 ppt are sometimes used to reduce both infectious diseases (e.g. parasites and fungi), as well as non-infectious diseases (*i.e.* “brown blood disease” caused by nitrite toxicity) in freshwater fish (Noga, 2010).

An emerging aquaculture candidate in Malaysia is the lemon fin barb hybrid (LFBH) that was first developed by the Malaysian Department of Fisheries in 2004 by crossing a male lemon barb *Hypsibarbus wetmorei* with a female silver barb *Barbodes gonionotus*. The LFBH combines the fast growth of *B. gonionotus* and the delicious taste of *H. wetmorei* and thus interest in LFBH within the aquaculture industry has rapidly spread with production increasing from approximately 29.93 tons in 2012 to 151.83 tons in 2014 (DOF, 2017). This has led to some investigations into the nutritional requirements of LFBH (Suharmili *et al.,* 2015; Ismail *et al.,* 2016), but there is no information on their optimal nursery conditions, such as salinity. However, it has been previously report, that *B. gonionotus* fry had significantly reduced growth at slightly elevated salinities of 3 ppt after 17 days (Romano *et al.,* 2017), while *H. wetmorei* are known to inhabit various rivers throughout South East Asia (Rim *et al.,* 2008; Zulkafli *et al.,* 2015). It would be intuitive that LFBH would also be more stenohaline, however, the performance of hybrids to salinity is variable. This has included lower salinity tolerances among various salmonid *Salmo* sp. and *Salvelinus* sp. crosses compared to their pure strains (Sutterlin *et al.,* 1977). On the other hand, tilapia *Oreochromis niloticus* and *O. mossambicus* hybrids had better growth over a broader salinity range than either parent (Kamal and Mair, 2005).

The aim of this study was to assess the survival and performance of LFBH to increasing salinities of 0, 3, 6 and 9 ppt, and subsequent influence to their whole-body histology and glycogen distribution.

Materials and Methods

Source of experimental animals : Mature male *Hypsibarbus wetmorei* and mature female *Barbodes gonionotus* were injected with Ovatide® at 0.5 and 0.8 ml kg^{-1}, respectively, and after 5 hrs the eggs and sperm were strip spawned and mixed for approximately 30 sec. The fertilized eggs were incubated in a 50 l aquarium that contained dechlorinated freshwater with gentle aeration. Within 24 hrs of hatching, the fry (full-siblings) were fed microworms (*Panagrellus redivivus*) for 3 days and then transferred to a 1,000 l fiberglass tank with gentle aeration. The fry were fed a commercial powdered food designed for tilapia (Dingding, Malaysia) to apparent for one month and each week approximately 20% of the water was exchanged.

Experimental design : A total of 180 apparently healthy fish (mean length and standard deviation of 1.3 ± 0.2 cm) were selected randomly from the acclimation tank and then equally distributed among twelve 10 l plastic aquaria. Each aquarium had gentle aeration and LFBH were fed to apparent satiation with commercial pellets designed for koi (Hikari Economy, Kyorin). During 3 days of further acclimation, the fish showed no apparent signs of stress. Tanks were randomly allocated one of the four salinity treatments of 0, 3, 6 and 9 ppt. Salinities were gradually raised with sea salt, naturally obtained from the ocean, to the desired level over 2 days. Salinity was determined using a hand refractometer (Iwaki, Japan), while temperature, dissolved oxygen and pH were measured daily using a digital multiprobe (YSI 1556, MPS).

Each morning (09:00) any uneaten food or faeces were siphoned out and then the fish were fed to satiation, and the fish were fed again in the afternoon (15:00), followed by siphoning again. During these times of siphoning, approximately 10% of the water was removed and then replaced each day and general observations were made for feeding activity and any signs of stress. Every week, 90% water exchange was performed in each replicate. Prior to each water exchange, the total ammonia-N and nitrite-N were measured using a test kit (TAN, API® freshwater master test kit, Chalfont, PA, USA). The pH, DO and temperature were 7.8 ± 0.2, 5.97 ± 0.1 mg l^{-1} and 27 ± 0.4°C, respectively. The ammonia-N levels were consistently below 0.2 mg l^{-1}, while nitrite-N was undetectable.

After 30 days, the fish were anaesthetized in clove oil and individually measured for their total length and weights using a digital caliper (Mitutoyo, Japan) and electronic balance, respectively. A total of six fish in each treatment were then immersion-fixed in Bouin’s solution for 8 hrs followed by transferring the fish to 70% (v/v) ethanol.

Whole-body histology : The fish were thoroughly rinsed in 70% ethanol over the course of 2 days, to remove any residual Bouin’s solution. The samples were then processed at increasing concentrations of ethanol, embedded in paraffin wax and then a total of 8 sections for each fish (6 µm thickness) were made using a rotary microtome. Half of these sections were stained with hematoxyling and eosin (H&E). Meanwhile, 2 sections (for each fish) were treated with α-amylase (Sigma Aldrich; 0.5%), while the other 2 received no pretreatment and then all were stained for Periodic-acid Schiff (PAS) was according to Romano *et al.* (2018). Sections were then mounted with distyrene plasticizer xylene (DPX) and a coverslip, and after sufficient drying, color digital pictures were obtained using a light microscope (Leica DM750; Imager ICC50).

Data analysis : The specific growth rates (SGR) for weights of fish in each treatment were calculated as follows:

SGR (weight) = ((LN(final weight) – LN(initial weight)) / (number of days)) X100

The condition factor (CF) of the fish in each treatment was determined by the following formula:

CF = (final weight)/(final total length^3) X100

The data was checked for normality (Shapiro-Wilks test) and homoscedasticity (Levene's test) before statistical analysis. After confirmation, a one-way ANOVA was performed on the growth data and if significant differences were detected ($p < 0.05$), a Tukey's post hoc test was used to identify the differences. A linear regression was also performed to determine the strength of the relationship between salinity and growth.

Results and Discussion

In the absence of controlled studies, it is generally assumed that freshwater fish may not survive or grow optimally at even slightly higher salinities during their early nursery culture. Indeed, survival and growth linearly decreased from 0 – 14 ppt in snakehead, *Channa striatus* fry (Amornsakun *et al.,* 2017) and 0 – 9 ppt in the freshwater catfish, *Heteropneustes fossilis* fingerlings (Ahmmed *et al.,* 2017) and *B. gonionotus* fry (Romano *et al.,* 2017). These fish species could, therefore, be described as stenohaline freshwater. In this study, LFBH had a more euryhaline characteristic. This is based on observing no mortality in any of the treatments over the 30-day experimental duration and, moreover, the LFBH had a significantly positive relationship between salinity

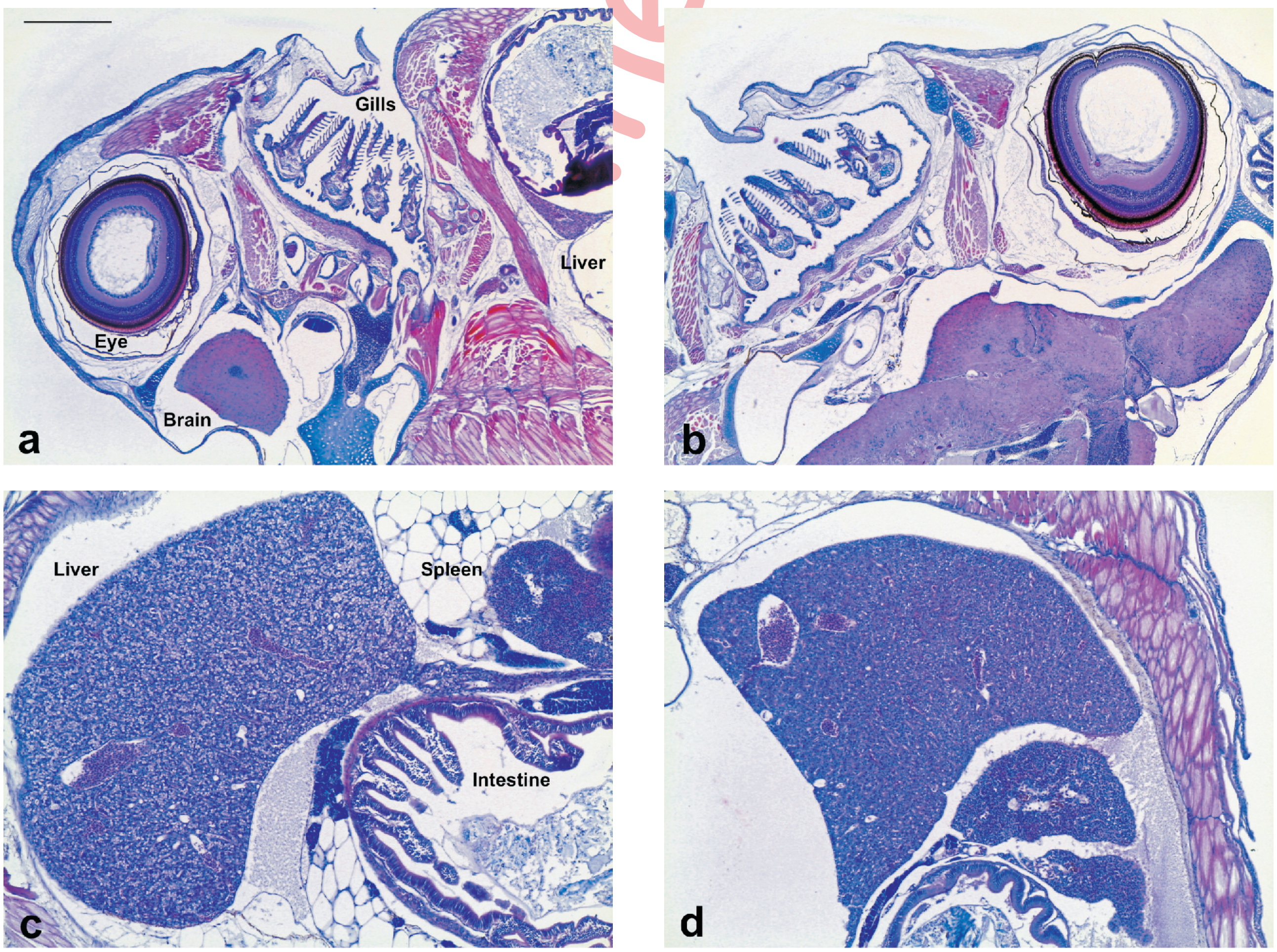


Fig. 1 : Sections of gills and surrounding tissue when lemon fin barb hybrids (*Hypsibarbus wetmorei* ♂ *Barbodes gonionotus* ♀) were subjected to (a) 0 ppt (b) or 9 ppt, as well as liver sections when fish were subjected to (c) 0 ppt (d) or 9 ppt . H&E staining; scale bar = 5 mm; magnification x 5

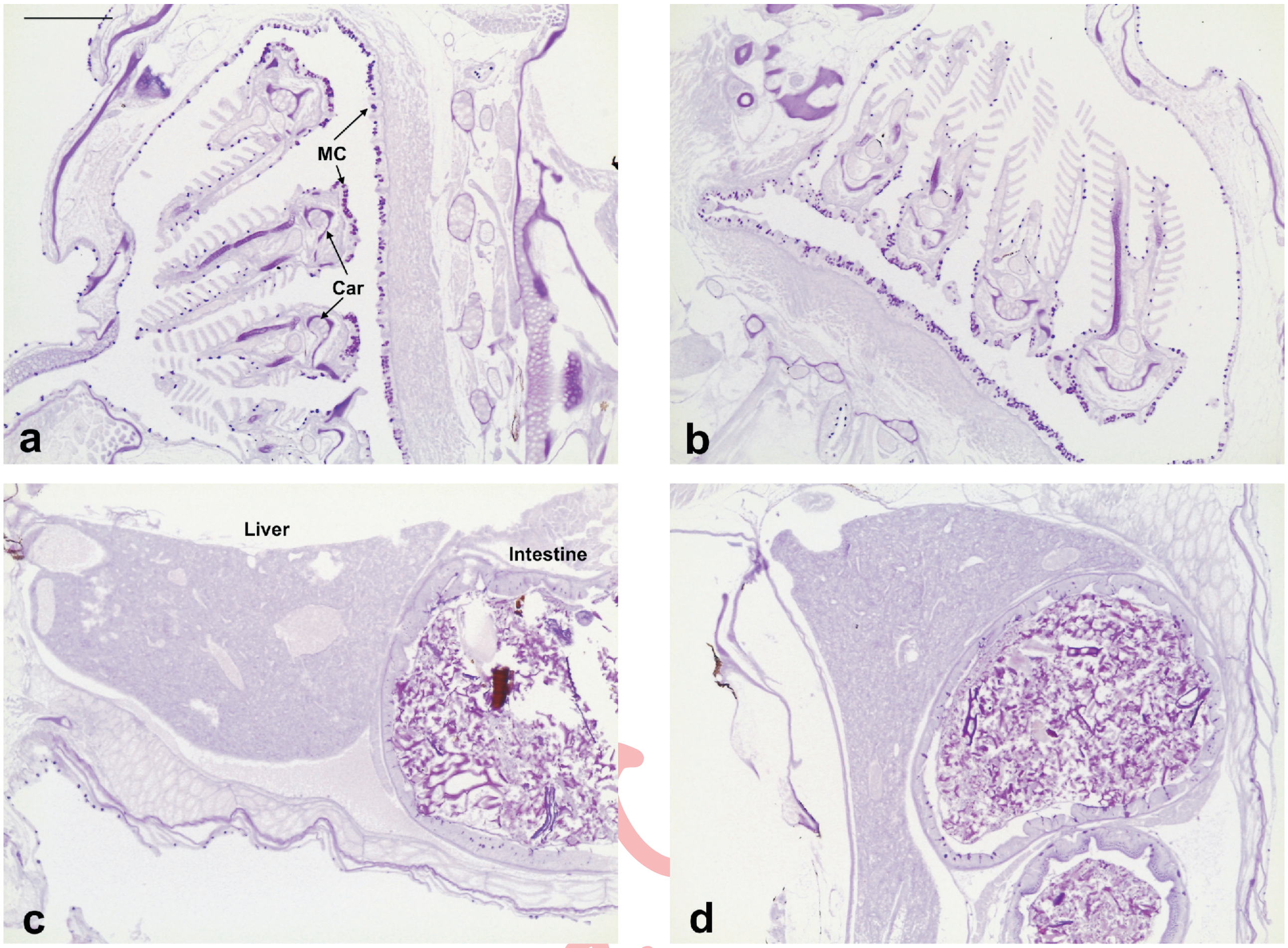


Fig. 2 : Sections of gills when lemon fin barb hybrids (*Hypsibarbus wetmorei* ♂ x *Barbodes gonionotus* ♀) were subjected to (a) 0 ppt (b) or 9 ppt, as well as liver sections when fish were subjected to (c) 0 ppt (d) or 9 ppt. Mucous cells (MC) and cartildge (Car). PAS staining; scale bar = 200 µm; magnification x 10

and SGR for weight ($p < 0.001$; $R^2 = 0.996$). The condition factor tended to decrease with increasing salinities, however, no significant relationship was found ($p = 0.316$; $R^2 = 0.01$).

The positive response of LFBH to increasing salinities indicate hybrid vigor, particularly because *H. wetmorei* are known to inhabit various freshwater rivers (Rim *et al.,* 2008; Zulkafli *et al.,* 2015). However, hybrid vigor cannot be totally confirmed until the performance of *H. wetmorei* is evaluated. For example, *O. niloticus* and *O. mossambicus* are known to grow better in lower and higher salinities, respectively, which likely explains the reason for their hybrid having faster growth in a wider salinity range than either parent (Kamal and Mair, 2005).

In addition to better growth of LFBH, there were no evidence of stress such as excessive gill mucous production, epithelial thickening of the secondary gill lamellae or liver vacuolization at increasing salinity conditions. This indicates that the tested salinities were well within the optimal range of LFBH because osmotic stress is generally associated with decreasing available energy, such as liver glycogen deposits (Sarma *et al.,* 2013; Ahmmed *et al.,* 2017). This is generally believed to be in response to increase energetic costs associated with osmoregulation and/or a disruption to metabolism (Febry and Lutz, 1987). The improved performance may be due to the tested salinities being closer to their iso-osmotic point along with a higher tolerance to salinity.

The mucous cells on the gills of fish produce mucous that can protect from water borne irritants, as well as reduce gill permeability and thus facilitate osmoregulation (Wilson and Laurent, 2002). Changes in the gill mucous cell prevalent to different salinity conditions appear to depends whether the fish is saltwater or freshwater adapted. For example, increasing salinities decreased the gill mucous cell prevalence in freshwater cave tetra *Anoptichthys jordani* (Mattheij and Sprangers, 1969) and *B. gonionotus* fry (Romano *et al.,* 2017). In contrast, seawater and brackish water acclimated Atlantic salmon *Salmo salar* and

Table 1 : Growth performance, condition factor and survival of lemon fin barb hybrids (*Hypsibarbus wetmorei* ♂ × *Barbodes gonionotus* ♀) to increasing salinities after 30 days

	0 ppt	3 ppt	6 ppt	9 ppt
Initial weight (mg)	19.60 ± 0.01	19.39 ± 0.04	19.13 ± 0.03	19.75 ± 0.01
Final weight (mg)	52.47 ± 1.09	54.44 ± 6.83	56.45 ± 4.32	58.10 ± 7.4
SGR weight	3.28 ± 0.07	3.36 ± 0.40	3.51 ± 0.25	3.57 ± 0.41
CF	1.93 ± 0.30	1.78 ± 0.14	1.56 ± 0.42	1.64 ± 0.21
Survival	100	100	100	100

guppy *Poecilia vivipara*, respectively, both showed a reduction to gill mucous cells after being transferred to freshwater (Roberts and Powell, 2003; Sabσia-Moraes *et al.,* 2011). A decrease in these mucous cells was suggested to compromise osmoregulation (Roberts and Powell, 2003; Romano *et al.,* 2017). Therefore, the unchanged mucous cell prevalent in LFBH further demonstrates that all the test salinities were not stressful to this fish.

The euryhalinity of LFBH can provide several advantages to their aquaculture in terms of site selection/husbandry, as well as disease management. For example, this may allow for polyculture with brackish water species such as shrimps because, in addition to their improved performance in brackish water, LFBH are non-aggressive. Indeed, polyculturing shrimp with euryhaline omnivores such as tilapia, *Oreochromis* sp. and mullet, *Mugil cephalus*, are sometimes performed in various countries as a way to maximize resources (Martvnez-Porchas *et al.,* 2010). Meanwhile, the use of slightly elevated salinities (1-3 ppt) during the early culture of freshwater fish may decrease the incidences of both non-infectious and infectious diseases (Noga, 2010). In the case of non-infectious diseases, salinities can reduce incidences of "brown blood disease" in fish, which is caused by excessive nitrite levels that bind with hemoglobin to form methemoglobin (Almendras, 1987).

This reason for this mitigating effect by salinity is because NO_2^- and Cl^- can substitute for each other during osmoregulation, and increasing salinities decrease the need to actively transport Cl^-, along with NO_2^-, inwards to the blood (Jensen, 2003). Meanwhile, some freshwater pathogens such as bacterium, *Flavobacterium columnare* are less effective at inducing fish mortalities at even modest salinity increase of 3 to 9 ppt (Altinok and Grizzle, 2001).

In conclusion, the euryhalinity of LFBH over the stenohaline characteristic of their parent *B. gonionotus* can potentially provide more flexibility to the husbandry of LFBH, such as polyculture with other brackish water shrimps. Moreover, the farming of LFBH can provide improved food security in areas experiencing increased salinization, which is becoming an increased concern in various parts of the world including Southeast Asia (Capedo-Argóelles *et al.,* 2016).

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