Acidification tolerance of *Barbonymus schwanenfeldii* (Bleeker, 1854) and *Oreochromis niloticus* (Linnaeus, 1758) – implication of fish size

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Abstract. The aim of the present study was to investigate the acidification tolerance of a Malaysian indigenous fish species, *Barbonymus schwanenfeldii* (Bleeker, 1854) and an exotic fish species, *Oreochromis niloticus* (Linnaeus, 1758). The survival rate, growth performance, and feed conversion ratio of fry and juvenile fish of *B. schwanenfeldii* and *O. niloticus* were tested in acidic water at pH 4.5, 5.5, and 6.5 for a period of 30 days. The fish reared in pH 7.0 acted as a control. The present study demonstrated that acidification in water reduced the survival rates of *B. schwanenfeldii* and *O. niloticus* and had a detrimental effect particularly on the survival of *B. schwanenfeldii* fry where a 100% mortality occurred under the exposure to pH 4.5. Additionally, the total length gain decreased significantly in the fry of both species when they were exposed to low pH water. The results also showed that acidification of water had more effect on fry than juvenile fish and acidification tolerance of the indigenous species *B. schwanenfeldii* was lower than the exotic species *O. niloticus*. The present study demonstrated the necessity to protect the indigenous species in Malaysia by maintaining a suitable water pH value in the natural habitat.
Key Words: acidic water pH, survival rate, growth performance, feed conversion ratio, fry and juvenile fish.

Introduction. Physicochemical water quality parameters affect the survival, growth, reproduction, and distribution of aquatic animals directly or indirectly (Patoine et al 2002; Freund & Petty 2007; Gupta et al 2012; Leduc et al 2013; Perkin & Bonner 2016). Among them, water pH plays a crucial role. Its roles and the effects of extreme water pH value on fish have been widely studied and reviewed (Underhay & Burka 1997; Ikuta et al 1999; Baldigo & Lawrence 2001; Scott et al 2005; Aride et al 2007; Henrique et al 2007; Oliveira et al 2008; Bolner et al 2014). It is well accepted that a suitable pH range for fish is 6.5 to 9.0 (Zweig et al 1999). However, different species have different pH tolerance and fish at different life stages also exhibits different responses to water pH value (Lloyd & Jordan 1964; Lopes et al 2001; Aride et al 2007; Ivoke et al 2007; Heydarnejad 2012). Such variations of fish tolerance to different pH indicate that pH changes in their natural habitat could greatly affect their survival and growth.

Malaysia has experienced a notable economic growth over the last few decades due to its industrialization and urbanization plan as well as land use changes for agriculture development that have resulted in degradation of the aquatic ecosystem (Khalik et al 2013; Gandaseca et al 2014; Low et al 2016). Anthropogenic activities such as agricultural runoff, deforestation, and sewer overflow all have major influence on water pH of the aquatic ecosystem. Low pH value (≤ 6.2) was observed in a river located downstream of a large hydroelectric dam in Sarawak (Ling et al 2016). A pH value as low as 5.2 was also recorded in a river that was subjected to the discharge of untreated municipal, industrial and agricultural wastes in Terengganu (Suratman et al 2015). Studies have shown that acidification of lakes and rivers often reduces the diversity and abundance of fish; low pH water value also increases the toxicity of pollutant to fish (Baldigo & Lawrence 2001; Henry et al 2001; Driscoll et al 2003; Schorr & Backer 2006; Baldigo et al 2016).

Barbonymus schwanenfeldii (Bleeker, 1854) which is usually known as "lampam sungai" in Peninsular Malaysia or "tengadak" in Sarawak is one of the important commercial indigenous fish species in Malaysia. Christensen (1992) stated that *B. schwanenfeldii* lives in lakes and rivers at pH range between 6.5 and 7.0. On the other hand, *Oreochromis niloticus* (Linnaeus, 1758), a cichlid fish native to central and North Africa and the Middle East is an exotic fish species in Malaysia. This species was introduced to the other parts of the world including Malaysia for farming purpose and became one of the world's most important food fishes (Fitzsimmons 2004; Chong et al 2010). *O. niloticus* is able to adapt to a wide range of environment due to its hardy nature. Numerous studies on pH tolerance have been conducted on *O. niloticus* (El-Sherif & El-Feky 2009; Cavalcante et al 2010; Rebouças et al 2016). Comparatively, the study of pH tolerance on *B. schwanenfeldii* is relatively scarce. Hence, this study aimed to examine the acidification tolerance of the indigenous fish species *B. schwanenfeldii* in comparison with the exotic fish species *O. niloticus*. The data would provide valuable insight on the response of *B. schwanenfeldii* when water acidification occurs in natural habitat.

Material and Method

Fish specimens. The fry and juvenile fish of *B. schwanenfeldii* and *O. niloticus* used in this study were obtained from the Department of Agriculture, Serian, Sarawak and PM Aquaculture Sdn. Bhd., Kota Sentosa, Sarawak, Malaysia, respectively. The fish were acclimatized for three days before the experiment. All the fish were active and no mortality occurred prior to the experiment. The initial total length (TL) and body weight (BW) of fry and juvenile fish of *B. schwanenfeldii* and *O. niloticus* were measured by using a digimatic caliper (Mitutoyo, CD-12'' CP) and a weighing balance (Adventurer, Dhaus), respectively. The mean values of initial TL of fry and juvenile *B. schwanenfeldii* were 1.50 cm and 3.51 cm, respectively while the mean values of initial TL of fry and juvenile *O. niloticus* were 1.26 cm and 3.30 cm, respectively. The mean values of initial BW of fry and juvenile *B. schwanenfeldii* were 0.05 g and 0.42 g, respectively while the mean values of initial BW of fry and juvenile *O. niloticus* were 0.03 g and 0.47 g, respectively.

Experimental design. The experiment was conducted from October to November 2013 for a period of 30 days. Five rectangular fiberglass tanks (2.1 m x 1.3 m x 0.6 m) filled with different water pH of 4.5, 5.5, 6.5 and 7.0 were used to determine the acidification tolerance of the fry and juvenile *B. schwanenfeldii* and *O. niloticus*. Anti-chlorine solution (1:5000 v/v) was added to each tank and they were aerated for three days before the experiment. The water pH was adjusted by adding sulfuric acid into the tank until a desired pH is obtained (Lopes et al 2001). Each tank was subdivided into smaller compartments (39 cm x 49 cm x 67 cm) made from polyvinyl chloride (PVC) frame and net with a capacity of 70 L water for each compartment. Fry and juvenile *B. schwanenfeldii* and *O. niloticus* were placed into each compartment for 30 days, in triplicate for each treatment. Each replicate consisted of twenty individuals of fish. The fish were fed twice daily at 5% body weight with pelleted fish feed throughout the experiment.

Temperature, pH, turbidity, and dissolved oxygen (DO) of each tank were measured weekly by using a pH meter with temperature probe (Hanna, HI8424), a turbidity meter (Eutech, TN-100), and a dissolved oxygen meter (Hanna, H19142), respectively. The experiment was conducted under a constant environment at approximately 25.5°C, DO value of 8.6 mg L^{-1}, and turbidity value of 0.05 FNU. Weekly water sample was also taken from each tank for ammonia-nitrogen (NH_3-N) and nitrite

(NO_2) analyses using Nessler method (HACH 2012). The mean concentrations of NH_3-N and NO_2 were 0.40 mg L^{-1} and 0.015 mg L^{-1}, respectively, throughout the experiment. Water was changed when the concentrations of NH_3-N and NO_2 were high. Daily fish mortality was recorded and dead fish was replaced immediately with fish receiving the same treatment to maintain the density of fish in each compartment. At the end of the experiment, the TL and BW of fish for each treatment were measured.
The acidification tolerance of fish was evaluated by using the survival rate, growth performance, and feed conversion ratio (FCR) calculated as:

$$\text{Survival rate (\%)} = N_i/N_0 * 100;$$
$$\text{Body weight gain (BWG)} = W_i - W_0;$$
$$\text{Total length gain (TLG)} = TL_i - TL_0;$$
$$\text{Specific growth rate (SGR)} = (\ln W_i - \ln W_0)/t * 100;$$
$$FCR = Df/(W_i - W_0).$$

Where N_i = number of fish at the end of the experiment, N_0 = initial number of fish, W_i = weight of fish at the end of the experiment (g), W_0 = initial weight of fish (g), TL_i = total length of fish at the end of the experiment (cm), TL_0 = initial total length of fish (cm), t = experiment time (day), Df = dry feed (g).

Statistical analysis. The survival rate, TLG, BWG, SGR, and FCR of fish exposed to different water pH were compared using the ANOVA test followed by Turkey test at p value < 0.05. All the statistical analyses were carried out by using the Statistical Software for Social Sciences (SPSS Version 22, SPSS Inc. 1995).

Results

Survival rate of fish. Figure 1 illustrates that survival rate of fry and juvenile fish of *O. niloticus* and *B. schwanenfeldii* was lower when water pH decreased. A significant lower ($p < 0.05$) survival rate of *B. schwanenfeldii* fry was observed at pH 5.5 (22%) and 4.5 (0%), whereas *B. schwanenfeldii* fry exposed to pH 4.5 died on the first day of the experiment. On the other hand, survival rates of fry and juvenile *O. niloticus* and juvenile *B. schwanenfeldii* were significantly lower ($p < 0.05$) as pH dropped to 4.5 level (55-82%). In contrast, when water pH was at 7.0, 100% survival rates were recorded for both species and life stages. The survival rate of *O. niloticus* fry was lower than juvenile as pH decreased but it was not statistically significant ($p > 0.05$). Overall, when water pH was 6.5 and below, the survival rate of *B. schwanenfeldii* fry was significantly lower than the other three categories, which are juvenile fish of the same species and that of different species ($p < 0.05$).

Growth performance of fish. Fry of *O. niloticus* and *B. schwanenfeldii* demonstrated significant lower in TLG ($p < 0.05$) from 1.33 cm to 0.59 cm and from 1.23 cm to 0.74 cm, respectively, when water pH decreased. Juvenile fish of both species also demonstrated similar trend but was not statistically significant ($p > 0.05$). There was no significant decrease ($p > 0.05$) in BWG and SGR of fish when water pH decreased. The BWG of fry (≈ 0.17 g) was significantly lower than juvenile fish (≈ 0.96 g) for both species at all pH treatments. The highest SGR was observed in *O. niloticus* fry (≈ 5.92%), followed by *B. schwanenfeldii* fry (≈ 4.79%). The SGR of *O. niloticus* fry was significantly higher ($p < 0.05$) than juvenile at pH 7.0 and 6.5 but was not significantly different at pH 5.5 and 4.5.

Feed conversion ratio. There was no significant difference ($p > 0.05$) in FCR of fish when water pH decreased as similar to BWG and SGR. The mean values of FCR of fry and juvenile *O. niloticus* and *B. schwanenfeldii* were 0.76, 1.46, 1.38, and 1.45, respectively. The FCR of *O. niloticus* fry was significantly lower ($p < 0.05$) than juvenile fish at water pH 7.0 but was not significantly different when water pH decreased to 6.5 and below.

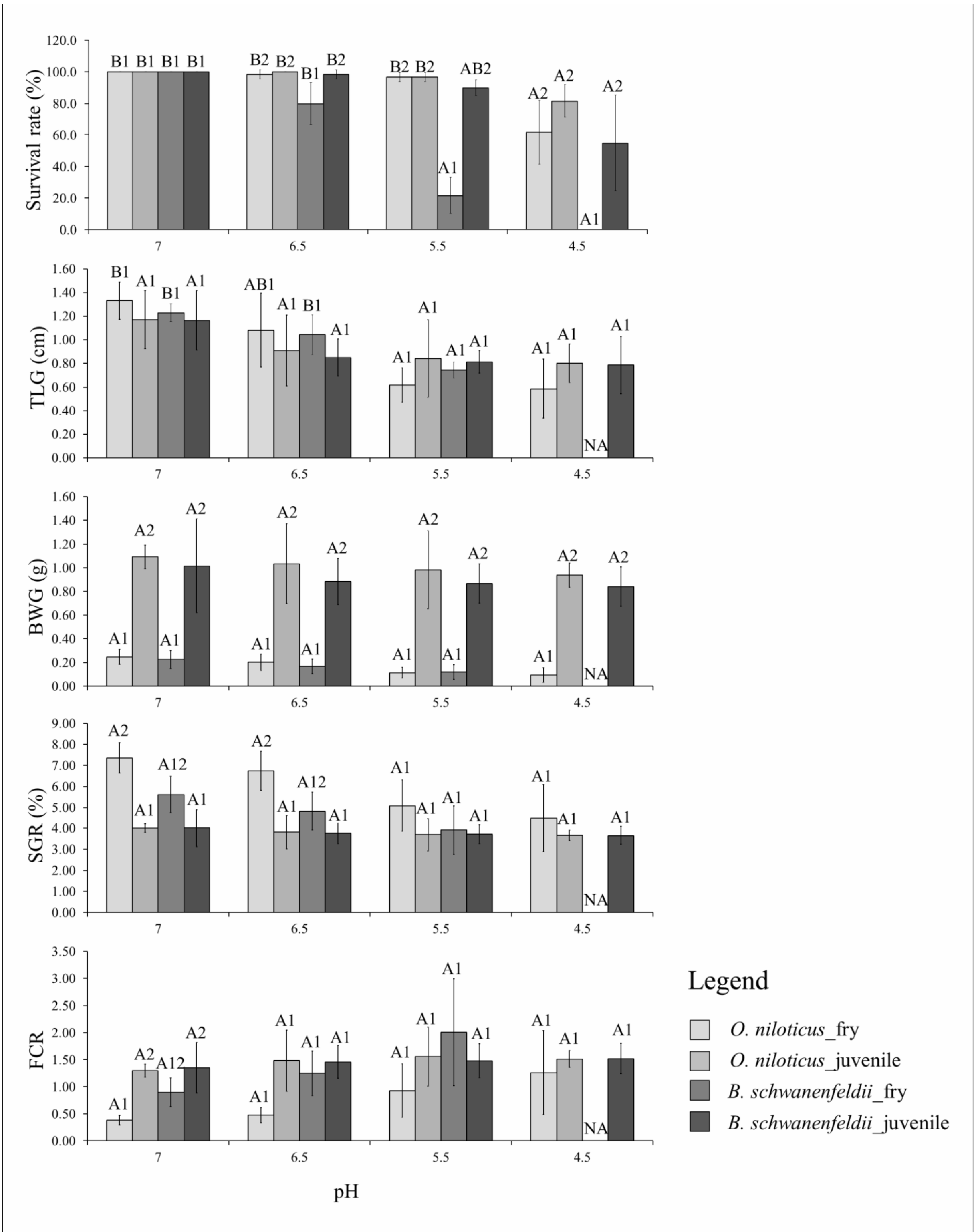

Figure 1. Survival rate, growth performance (TLG, BWG, and SGR), and FCR of fry and juvenile *Oreochromis niloticus* and *Barbonymus schwanenfeldii* subjected to different water pH for 30 days. Different letters indicate significant difference between pH treatments whereas different numbers indicate significant difference between fish at p < 0.05. NA = Not applicable as 100% mortality occurred.

Discussion. The present study demonstrated that acidification in water reduced the survival rates of *O. niloticus* and *B. schwanenfeldii* and had a detrimental effect particularly on the survival of *B. schwanenfeldii* fry where only 22% of them survived when they were exposed to water pH 5.5. Moreover, a 100% mortality of *B.*

schwanenfeldii fry occurred when it was exposed to water pH 4.5. The fish death could be due to the respiratory failure or osmoregulation failure to maintain ion balance (Gonzalez et al 1997; Matsuo & Val 2002; Duarte et al 2013). The juvenile fish of *B. schwanenfeldii* was more tolerant to low pH water than fry where more than 90% and 55% of juvenile fish survived when it was exposed to pH 5.5 and 4.5, respectively. The indigenous species *B. schwanenfeldii* is more susceptible to acidic condition when compared to the exotic species *O. niloticus*. Both fry and juvenile of *O. niloticus* can tolerate low water pH value up to 5.5 where nearly all fish (≈ 97%) survived throughout the experiment at this pH value. Similarly, the juvenile of *O. niloticus* survived better than fry where 82% of juvenile fish survived compared to 62% of fry surviving in pH 4.5 water.

The present study showed that *O. niloticus* and *B. schwanenfeldii* fry and juvenile fish exhibited stronger acidification tolerance than common carp, *Cyprinus carpio* and silver catfish, *Rhamdia quelen*. Heydarnejad (2012) reported that 4 to 11% of common carp survived exposure to acidic waters with pH value ranging from 6.0 to 6.5 while 100% mortality occurred when silver catfish larvae was exposed to pH 5.5 water (Lopes et al 2001). On the other hand, *O. niloticus* and *B. schwanenfeldii* fry and juvenile fish were less acidification tolerant than juvenile tambaqui, *Colossoma macropomum* (Aride et al 2007) and cardinal tetra, *Paracheirodon axelrodi* (Oliveira et al 2008) where no mortality was observed when they were exposed to water pH 4. The authors also reported that the 96-h LC_{50} of cardinal tetra to acidic pH was 2.9 showing that cardinal tetra is highly tolerant to acidic water pH.

Exposure of fish to low pH water also influenced the growth performance of *O. niloticus* and *B. schwanenfeldii* particularly TLG of fry in the present study. The TLG and BWG of fish were inversely proportional to the pH decrease. It was observed that there were 56% and 62% decreases in the TLG and BWG of *O. niloticus* fry when it was exposed to pH 4.5 compared to pH 7.0. *B. schwanenfeldii* fry also decreased up to 40% and 47% in TLG and BWG when they were exposed to pH 5.5 water compared to pH 7.0. However, the decrease was only statistically significant ($p < 0.05$) for TLG in the present study. Similar results were also reported previously by El-Sherif & El-Feky (2009) and Heydarnejad (2012).

The present study demonstrated that there were no significant differences ($p > 0.05$) in BWG, SGR, and FCR of *O. niloticus* and *B. schwanenfeldii* when water pH decreased. Similarly, Ivoke et al (2007) reported no significant difference in FCR of hybrid catfish juvenile. However, the present study results were in contrast with other studies which demonstrated that SGR and FCR were influenced by the pH value (El-Sherif & El-Feky 2009; Faramarzi et al 2011). Nevertheless, we did notice that in normal pH condition, the BWG of fry was significantly lower than the juvenile stage. The SGR of fry was significantly higher whereas the FCR of fry was significantly lower than juvenile fish. However, the significant difference of SGR and FCR between fry and juvenile fish disappeared in acidic conditions. This is most probably due to the lower growth rate and higher FCR in fry due to the more acidic condition. The result demonstrated that acidification in water had more effect on SGR and FCR of fry than that of juvenile fish.

Previous studies demonstrated that the pH tolerant level for *O. niloticus* (Ross 2000) and *B. schwanenfeldii* (Huwoyon et al 2010) ranged from 3.7 to 11 and from 5 to 7, respectively. Recently, Rebouças et al (2016) reassessed the suitable range of water pH for culture of Nile tilapia, *O. niloticus* in eutrophic water. The authors concluded that the suitable range of water pH for rearing Nile tilapia is within 5.5-9.0 as pH 5.5 has no negative influence on growth of Nile tilapia fingerlings in eutrophic tanks. However, fish size needs to be taken into consideration in the evaluation of acidification tolerance of fish as fish is generally more sensitive when exposed to pH changes at early stages (Lloyd & Jordan 1964). Rask (1984) compared the sensitivity between different stages of fish and concluded that the pH tolerance increased with the age of the fish. Taghizadeh et al (2013) demonstrated that high mortality of the yolk-sac fry of goldfish (*Carassius auratus gibelio*) occurred in low pH water where the highest mortality was observed in pH 5.75 after three days.

The present study demonstrated that the indigenous species *B. schwanenfeldii* fry has low survival rate in acidic condition, and hence the fish is vulnerable in the natural environment facing water acidification problem due to anthropogenic activities. Fish species with low acidification tolerance tends to be eliminated while species that tolerate better in acidic water may proliferate. The lower acidification tolerance of the indigenous species *B. schwanenfeldii* when compared to the exotic species *O. niloticus* indicated the incapability of Malaysian indigenous fish species to survive in a deteriorated environment when compete with those hardy exotic fish species. This study suggests the necessity in the management and conservation of forest streams and rivers particularly the water pH to prevent possible losses of the indigenous fish species in Malaysia.

Conclusions. The present study demonstrated that acidification tolerance of the Malaysian indigenous species *B. schwanenfeldii* was lower than the exotic species *O. niloticus*. The tolerance of both fish species was influenced by fish size whereby fry stage was more susceptible to low pH water than juvenile stage of the fish. The impact of water acidification had the most detrimental effect on *B. schwanenfeldii* fry. Acidification of water reduced survival rates of *O. niloticus* and *B. schwanenfeldii* in both fry and juvenile stages. The TLG of fry of both fish species was found significantly lower in more acidic water condition. Neither fry nor juvenile stage of the two fish species showed significant difference on BWG, SGR, and FCR in increasing acidic condition.

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