Effects of water temperature and pH on total suspended solids tolerance of Malaysian native and exotic fish species

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Abstract. The aim of the present study was to investigate the effects of water temperature and pH on total suspended solids (TSS) tolerance of a native fish species, *Barbonymus schwanenfeldii* (Bleeker, 1854) and an exotic fish species, *Oreochromis niloticus* (Linnaeus, 1758) in Malaysia. The survival rates of both juvenile fish were tested in different TSS concentrations (0, 500, 1000, and 5000 mg L^{-1}) at two water temperatures (27 and 29 °C) and three water pH (pH 5, 6, and 7) for 21 days. The results showed that biotic and abiotic factors significantly influenced fish survival rate. The survival rate of juvenile fish significantly ($p < 0.05$) decreased when they were exposed to warm and low pH water and high suspended solids. Moreover, the TSS tolerances of both juvenile fish in term of survivorship exacerbated at warm and low pH water. The *O. niloticus* juveniles were significantly ($p < 0.05$) more tolerant to low water pH and high TSS than the *B. schwanenfeldii* juveniles. Nevertheless, the warmer water did not affect survival rate of *B. schwanenfeldii* juveniles significantly ($p < 0.05$) more than that of *O. niloticus* juveniles. The present study demonstrated the potential negative impacts of water quality deterioration and biological invasion on the survival of Malaysian native fish in natural environment.

Key Words: survival rate, combined effect, acidification, sedimentation, biological invasion.

Introduction. Recently, the problem of deforestation and degradation of forests in Sarawak, Malaysia is giving rise to some concern (Hon & Shibata 2013). Majority of the surface cover of the state is categorized as forested land which has been impacted by previously undocumented and high intensity logging activities. Deforestation is often associated with sedimentation problem which leads to high suspended solids and turbidity in freshwater ecosystem (Gomi et al 2006). Canopy removal and the high levels of total suspended solids (TSS) will increase water temperatures due to direct solar radiation and heat absorption by suspended particles (Moore et al 2005; Ling et al 2016). Also, freshwater ecosystems are susceptible to acidification by acid rain, nutrient runoff, and other anthropogenic activities (Tetzlaff et al 2007; Gravelle et al 2009).

Physicochemical water quality parameters affect directly or indirectly fish survival and determine the distribution and abundance of fish in freshwater ecosystem (Freund & Petty 2007; Bilotta & Brazier 2008; Gupta et al 2012; Kjelland et al 2015). Since environmental degradation rarely experience single variable deterioration, aquatic organisms are forced to cope with multiple environmental stresses. Interactions of those stressors on fish can sometimes result in greater effects than expected from either of the stressor alone. The interactive effects between various stressors and toxicants on fish are not uncommon phenomena (Sappal et al 2014; Di Santo 2015; Grasset et al 2016). However, most laboratory studies often expose test organisms under optimal environmental conditions to test the influence of particular stressor on fish survival. Currently, little is known about the combined effects of various stressors including the combined effects of suspended solids and water temperature as well as suspended solids and water pH on fish survival.

In addition to the environmental degradation, freshwater ecosystems can be impacted by invasive species which are highly competitive and can tolerate abroad wide range of environmental conditions (Hellmann et al 2008; Butler et al 2010; Leuven et al 2011). The biological invasion can exaggerate the combined effects posed by water quality deterioration and invasive species on native fish populations. A study of the influences of various water quality changes on the survival of native and exotic fish species is vital to effectively forecast the effects of environmental degradation and biological invasions on freshwater ecosystem. Hence, this study aims to study the iterative effects of TSS and water temperature as well as TSS and water pH on survival of the native fish species *B. schwanenfeldii* and compared with the exotic fish species *O. niloticus* in Malaysia.

Material and Method

Fish specimens. The *B. schwanenfeldii* and *O. niloticus* juveniles were obtained from the Department of Agriculture, Serian and PM Aquaculture Sdn. Bhd., Kota Sentosa, respectively. The fishes were acclimatized for a week before the experiment. All the fishes were active and no mortality occurred prior to the experiment. The initial total length (TL) and body weight (BW) of *B. schwanenfeldii* and *O. niloticus* juveniles were measured to the nearest 0.1 millimeter and to the nearest 0.01 gram by using a digital caliper (Mitutoyo, D15TN) and a weighing balance (AND, GH-252), respectively. The mean values of initial TL and BW of *B. schwanenfeldii* and *O. niloticus* juveniles at each experiment were summarized in Table 1. Juvenile stage of fish was selected in the present study. Since the temperature and pH experiments were two independent experiments, the fishes used in each experiment were chosen as long as they were in juvenile stage.

Experimental design. Two experiments were conducted in the present study. The experiments were conducted from October to December 2014 in the External Laboratory of Universiti Malaysia Sarawak, Malaysia. In experiment 1, *B. schwanenfeldii* and *O. niloticus* juveniles were exposed to water containing different concentrations of TSS at two water temperatures of 27 and 29°C. In experiment 2, the juvenile fishes were exposed to water containing different concentrations of TSS coupled with three water pH of 5, 6, and 7. Sediment sample collected from the banks of Sarawak River near Kampung Landeh was used to prepare different TSS concentrations. A selected weight of the sediment was added into each tank to attain TSS concentrations of 500, 1000, and 5000 mg L^{-1}. Tank without sediment addition was served as a control. TSS was determined as the difference between the initial and final weight of glass fibre filter, after filtration of an adequate sample volume and drying at 105°C. The water temperature was adjusted by using a thermostatic heater (Tianrum, W-500HG) while the water pH was adjusted by adding sulfuric acid into the tank until a desired pH is obtained (Lopes et al 2001).

Rectangular fiberglass tanks (2.1 m x 1.3 m x 0.6 m) which subdivided into smaller compartments (0.4 m x 0.4 m x 0.6 m) that made from polyvinyl chloride (PVC) frame and net were used in the present study. Each tank was filled with 1.15 m^3 of tap water and aerated for a week prior to the experiment. Each compartment in the tank has a water capacity of 0.096 m^3. *B. schwanenfeldii* and *O. niloticus* juveniles were placed into each compartment for 21 days. All treatments were conducted in triplicate where one compartment of a tank serves as one replicate. Each replicate consisted of twenty individuals of a single fish species. The fish were fed twice daily at 5% body weight with pelleted fish feed containing 40% of protein throughout the experiment.

Water temperature, pH, and dissolved oxygen (DO) of each tank were measured daily by using a pH meter with temperature probe (HORIBA, D-51E) and a DO meter (SPER SCIENTIFIC, 850041), respectively. Weekly water sample was also taken from each tank for total ammoniacal nitrogen (TAN) and nitrite (NO_2) analyses by using Nessler method and colorimetric diazotization method, respectively (Hach 2015). All water quality parameters, except for the studied parameter, were maintained as constant value throughout the experiment as summarized in Table 1. Daily fish mortality was recorded and dead fishes were replaced immediately with fishes receiving the same treatment to maintain the density of the fishes in each compartment. The survival rate of the fishes was calculated as:

$$\text{Survival rate (\%)} = Ni/N0*100.$$

where Ni = number of fish at the end of the experiment, N0 = initial number of fish.

Statistical analysis. A three-way ANOVA test was used to study the interactions of fish species, TSS concentration, and water temperature on survival rate of the fishes, followed by the Turkey test at p value < 0.05. The interactive effects of fish species, TSS concentration, and water pH on survival rate of the fishes were also analysed using the three-way 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

Interactive effects of water temperature and total suspended solids on survival rates of Malaysian native and exotic fish species. Figure 1 illustrates the survival rates of *B. schwanenfeldii* and *O. niloticus* juveniles that were subjected to different TSS concentrations at 27 and 29°C. No mortality of fishes was observed when they were subjected to 0 mg L^{-1} of TSS at both water temperatures. The survival rates of the fishes were significantly lower (p value < 0.05) when TSS concentration and water temperature were increased. The survival of *B. schwanenfeldii* juvenile decreased from 100% to 52 and 47% at 27 and 29°C, respectively, when TSS concentration was increased from 0 to 5000 mg L^{-1}; while the survival of *O. niloticus* juvenile decreased from 100% to 63 and 53% at 27 and 29°C, respectively when TSS concentration was increased from 0 to 5000 mg L^{-1}. The survival of Malaysian native fish species, *B. schwanenfeldii* was significantly ($p < 0.05$) lower than the exotic fish species, *O. niloticus*. There was a significant interaction ($p < 0.05$) between fish species and TSS concentration on survival; but fish species and water temperature had no significant interactive effect on fish survival ($p > 0.05$) (Table 2). The decrease of the fishes survival rate as TSS concentration increase was greater for *B. schwanenfeldii* juvenile than *O. niloticus* juvenile whereas the effect of warmer water was the same on survival rates of *B. schwanenfeldii* juvenile and *O. niloticus* juvenile. TSS concentration and water temperature had a significant interactive effect on the fishes survival rate ($p < 0.05$). Higher water temperature leads to a decrease of the fishes survival rate as TSS concentration increased. There was no statistically significant ($p > 0.05$) three-way interaction between fish species, TSS concentration, and water temperature.

Interactive effects of water pH and total suspended solids on survival rates of Malaysian native and exotic fish species. Figure 2 illustrates that all the fishes survived when they were exposed to TSS concentration of 0 mg L^{-1} at pH 7. The survival rate of the fishes were directly proportional to water pH and inversely proportional to TSS concentration. TSS concentration and water pH had a significant interactive effect on the fishes survival rate ($p < 0.05$) as shown in Table 3. As TSS concentration increase, the survival rate of *B. schwanenfeldii* juvenile decreased from 100% to 70%, 92% to 48%, and 82% to 27% at water pH 7, pH 6, and pH 5, respectively. Similarly, the survival rate of *O. niloticus* juvenile decreased from 100% to 85%, 95% to 67%, and 87% to 52% at water pH 7, pH 6, and pH 5, respectively. The effects of water pH was much more profound on the survival rate of Malaysian native fish species, *B. schwanenfeldii* than the exotic fish species, *O. niloticus* as indicated by significant interaction ($p < 0.05$) between fish species and water pH on survival rate. There was no statistically significant ($p > 0.05$) three-way interaction between fish species, TSS concentration, and water temperature on survival.

Table 1

Summary of the environmental conditions, initial total length, and initial body weight of fish used in the present study

TSS (mg L^{-1})	*Parameter*	*Temperature (°C)*		*pH*		
		27	*29*	*5*	*6*	*7*
0	Temperature (°C)	-	-	25.9±0.9	25.9±0.9	25.9±0.9
	DO (mg L^{-1})	7.5±0.0	7.5±0.0	7.7±0.3	7.7±0.3	7.7±0.3
	pH	7.2±0.0	7.2±0.0	-	-	-
	TAN (mg L^{-1})	0.78±0.24	0.77±0.06	0.81±0.05	0.81±0.05	0.81±0.05
	NO_2-N (mg L^{-1})	0.01±0.01	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.01
500	Temperature (°C)	-	-	25.9±0.9	25.9±0.9	25.9±0.9
	DO (mg L^{-1})	7.5±0.0	7.5±0.0	7.7±0.3	7.7±0.3	7.7±0.3
	pH	7.1±0.0	7.1±0.0	-	-	-
	TAN (mg L^{-1})	0.72±0.06	0.80±0.07	0.81±0.05	0.81±0.05	0.81±0.05
	NO_2-N (mg L^{-1})	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
1000	Temperature (°C)	-	-	25.9±0.9	25.9±0.9	25.9±0.9
	DO (mg L^{-1})	7.5±0.0	7.5±0.0	7.7±0.3	7.7±0.3	7.7±0.3
	pH	7.1±0.0	7.1±0.0	-	-	-
	TAN (mg L^{-1})	0.71±0.08	0.65±0.05	0.81±0.05	0.81±0.05	0.81±0.05
	NO_2-N (mg L^{-1})	0.01±0.00	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.01
5000	Temperature (°C)	-	-	25.9±0.9	25.9±0.9	25.9±0.9
	DO (mg L^{-1})	7.5±0.0	7.5±0.0	7.7±0.3	7.7±0.3	7.7±0.3
	pH	7.1±0.0	7.1±0.0	-	-	-
	TAN (mg L^{-1})	0.76±0.04	0.69±0.07	0.81±0.05	0.81±0.05	0.81±0.05
	NO_2-N (mg L^{-1})	0.01±0.00	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.01
Barbonymus schwanenfeldii						
Initial TL (mm)		30.1±3.0			35.5±3.0	
Initial BW (g)		0.41±0.23			0.54±0.23	
Oreochromis niloticus						
Initial TL (mm)		33.7±2.9			37.7±2.9	
Initial BW (g)		0.67±0.11			0.84±0.14	

TAN: total ammoniacal nitrogen.

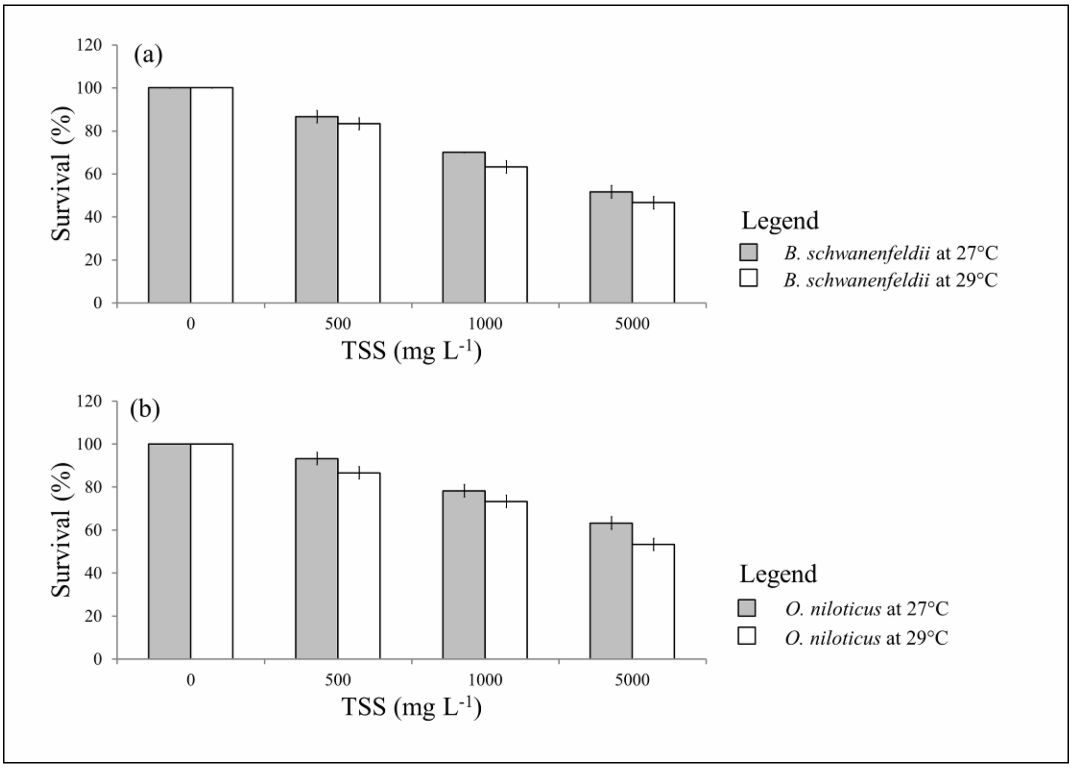

Figure 1. Survival rates of juvenile (a) *Barbonymus schwanenfeldii* and (b) *Oreochromis niloticus* subjected to different TSS concentrations at 27°C and 29°C for 21 days.

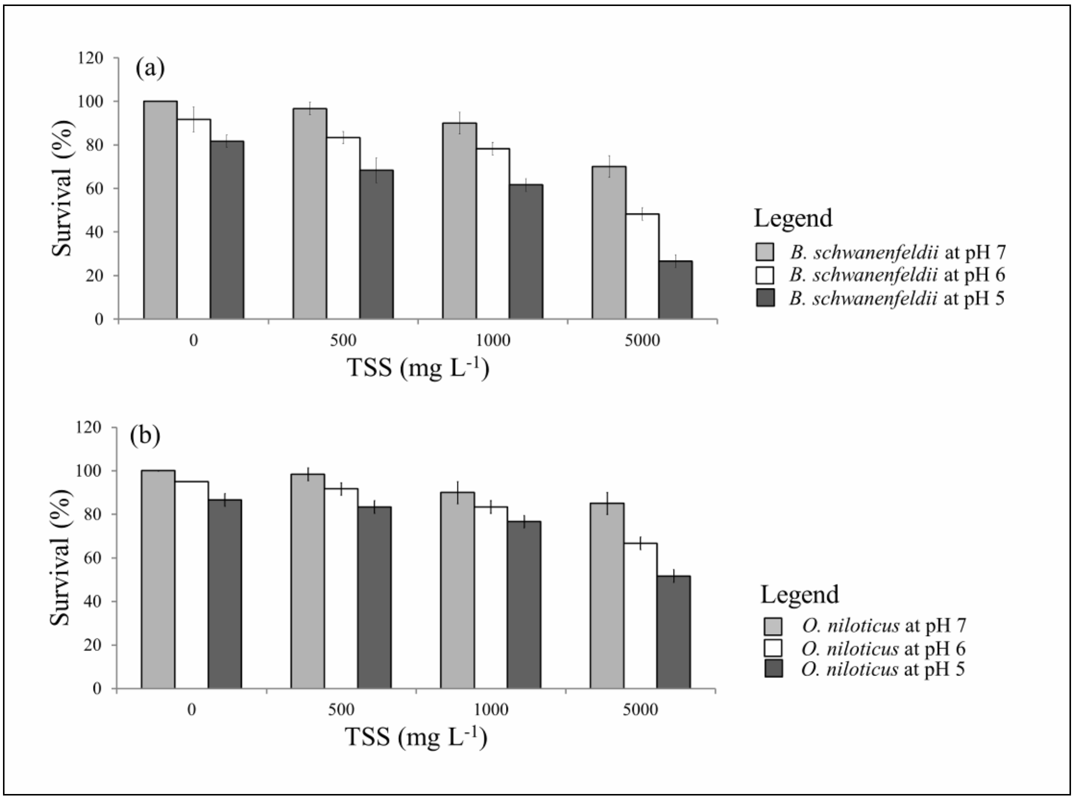

Figure 2. Survival rates of juvenile (a) *Barbonymus schwanenfeldii* and (b) *Oreochromis niloticus* subjected to different TSS concentrations at water pH 5, 6, and 7 for 21 days.

Table 2

Significance of fish species (Malaysian native and exotic), water temperature (27 and 29°C), and TSS concentration (0, 500, 1000 and 5000 mg L^{-1}) and their interactions on fish survival analyzed using Three-way ANOVA

Source of variation	*Type III sum of squares*	*df*	*Mean square*	*F*	*p value*
Fish species	408.333	1	408.333	71.273	**0.000**
Water temperature	252.083	1	252.083	44	**0.000**
TSS concentration	14493.75	3	4831.25	843.273	**0.000**
Interaction of fish species and water temperature	8.333	1	8.333	1.455	0.237
Interaction of fish species and TSS concentration	170.833	3	56.944	9.939	**0.000**
Interaction of water temperature and TSS concentration	93.75	3	31.25	5.455	**0.004**
Interaction of fish species, water temperature, and TSS concentration	20.833	3	6.944	1.212	0.321
Error	183.333	32	5.729		
Corrected total	15631.25	47			

Significant p value was indicated as bold.

Table 3

Significance of fish species (Malaysian native and exotic), water pH (pH 5, 6, and 7), TSS concentration (0, 500, 1000 and 5000 mg L^{-1}) and their interactions on fish survival analyzed using Three-way ANOVA

Source of variation	*Type III sum of squares*	*df*	*Mean square*	*F*	*p value*
Fish species	1558.681	1	1558.681	128.257	**0.000**
Water pH	7014.583	2	3507.292	288.6	**0.000**
TSS concentration	12320.486	3	4106.829	337.933	**0.000**
Interaction of fish species and water pH	354.861	2	177.431	14.6	**0.000**
Interaction of fish species and TSS concentration	689.931	3	229.977	18.924	**0.000**
Interaction of water pH and TSS concentration	874.306	6	145.718	11.99	**0.000**
Interaction of fish species, water pH, and TSS concentration	50.694	6	8.449	0.695	0.655
Error	583.333	48	12.153		
Corrected Total	23446.875	71			

Significant p value was indicated as bold.

Discussion. The present study shows a significant effect of various stressors on the fishes survival. When exposed to warm and low pH water with high suspended solids, *B. schwanenfeldii* and *O. niloticus* juveniles exhibit decreased survival. Statistical analysis revealed that abiotic factors including water temperature, water pH, and TSS had a significant effect on the fishes survival rate ($p < 0.05$). Survival of *B. schwanenfeldii* and *O. niloticus* juveniles were negatively affected by TSS exposure, experiencing reduced survival rate (≈ 58%) when exposed to 5000 mg L^{-1} of TSS. The survival rate of both juvenile fishes also significantly reduced to 67% when they were exposed to water pH 5. Warmer water per se, also affected the fishes survival, but in a lesser extent where increased water temperature of 2°C reduced the survival rate of the fishes from 80% to 76%. Furthermore, significant interactions were detected between water temperature and TSS as well as water pH and TSS ($p < 0.05$). The joint effect of warmer water and TSS exposure significantly ($p < 0.05$) decreased the survival rate of the fishes to 50% at 29°C and 5000 mg L^{-1} of TSS. Under the combined effect of acidification and TSS exposure, the survival of the fishes was decreased even more (≈ 39%) at pH 5 and 5000 mg L^{-1} of TSS.

Exposure of the fishes to high suspended solids can cause physical damage to gill structure of fish and clogging which leads to respiratory failure and mortality (Au et al 2004). The effects of suspended solids on fish survival differ across species depending upon species-specific tolerance of suspended solids. The suspended sediment mortality effects for several fish species had also been reviewed (Kjelland et al 2015). Our results corroborate the negative effect of suspended solids on juvenile fish survival. The fish survival was negatively proportional to TSS concentration. Similar result was also presented by Buermann et al (1997). Furthermore, the effect of suspended solids exposure has been shown on fish eggs where eggs of smallmouth bass (*Micropterus dolomieu*) that exposed to more than 100 mg L^{-1} of TSS had resulted in decreased survival of post-hatch larval fish (Suedel et al 2017).

Rising water temperature is increasingly recognized as a potential stressor for tropical ectotherms which are predicted to have a narrower thermal tolerance as compared to temperate ectotherms (Chrétien & Chapman 2016). The present study demonstrated that the fishes survival significantly ($p < 0.5$) decreased as water temperature increased corroborate the thermal stress on fish. In addition, there is a growing body of literature suggesting that thermal stress may influence how aquatic organisms react to other biotic and abiotic stressors (Gallardo & Aldridge 2013; Sappal et al 2014; Grasset et al 2016; Mueller et al 2017; Sampaio et al 2017; Cardoso et al 2018). Our data also support that the combination of temperature stress and TSS exposure exacerbates the survival of the fishes that these stressors alone induce. Similarly, Grasset et al (2016) demonstrated that the combination of temperature stress and metal exposure also modified the response of antioxidant metabolism in yellow perch (*Perca flavescens*) induced by these stressors individually. It is hypothesized that temperature exacerbated the effects of metal by lowering the concentration of the metal required for toxicity (Sappal et al 2014). The authors demonstrated greater bioenergetic disturbances during concurrent Cu and thermal stress on mitochondria isolated from rainbow trout livers. Mueller et al (2017) studied the effects of temperature, total dissolved solids, and total suspended solids on the developmental and survival rates of Arkansas River Shiner larvae. Those embryos incubating at high temperatures, or in high levels of total dissolved solids and total suspended solids resulted in less viable embryos and larvae than those incubating in all other treatment groups.

The pH plays an important role in homeostasis of fish where pH changes may induce ion disruption and ammonia excretion in fish (Matsuo & Val 2002; Scott et al 2005; Aride et al 2007). Previous study had demonstrated that acidification in water reduced the survival rates of *B. schwanenfeldii* and *O. niloticus* (Nyanti et al 2017). The present study further proved that combination of low water pH and high TSS exposure exacerbates the survival of the fishes. The interactive effects of pH and other stressors have been well documented. Miron et al (2008) demonstrated that survival of juvenile silver catfish exposed to different ammonia levels was altered by pH value. The combination of low pH and high Al is more toxic to larval goldfish as solubility of Al increases in acidic condition (Taghizadeh et al 2013). Nevertheless, Haddaway et al (2013) demonstrated that both pH and *Thelohania contejeani* infection affected the survival of the British white-clawed crayfish (*Austropotamobius pallipes*) but there was no interaction between *T. contejeani* infection and pH on crayfish survival.

Biotic factor such as biological invasion is one of the pervasive components of the global changes, particularly of the biodiversity changes in freshwater ecosystem (Sala et al 2000; Simberloff et al 2013). The present study demonstrated that the Malaysian native species *B. schwanenfeldii* juvenile was more susceptible to high TSS and low water pH than the exotic species *O. niloticus* juvenile as indicated by a significant interaction ($p < 0.05$) between fish species and TSS concentration as well as fish species and water pH on their survival rate. Surprisingly, the warmer water did not affect survival rate of *B. schwanenfeldii* juvenile significantly more than that of *O. niloticus* juvenile as fish species and water temperature had no significant interactive effect on fish survival rate ($p > 0.05$). Leuven et al (2011) demonstrated that the interspecific variation in the temperature tolerance of exotic fish species was found to be larger than the native species in the Rhine River at both ends of the temperature range. The larger thermal tolerance of the exotic fish species than that of native fish species had prevented the full recovery of native fish species in the river. On the other hand, Gallardo & Aldridge (2013) demonstrated that although both native and invasive crayfishes in Europe were predicted to be negatively affected by climate changes, the gradual contraction over time in their geographic range size was more severe for the invasive signal crayfish (*Pacifastacus leniusculus*). Moreover, the overlap between both crayfishes decreased by 13-16%, which may help to reduce the pressure upon the native white-clawed crayfish (*Austropotamobius pallipes*) in Europe.

Conclusions. The present study demonstrated that when exposed to high suspended solids, coupling with warming or acidification, *B. schwanenfeldii* and *O. niloticus* juveniles exhibited decreased survival. The combined effect of warmer water and TSS exposure as well as low pH water and TSS exposure significantly ($p < 0.05$) decreased the survival rate of the fishes more than these stressors alone induce. The Malaysian native species *B. schwanenfeldii* juvenile was more susceptible to high TSS and low water pH than the exotic species *O. niloticus* juvenile; yet water temperature had no significant interactive effect on the fishes survival rate ($p > 0.05$).

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