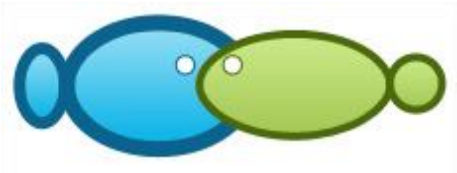

# Tolerance of *Barbonymus schwanenfeldii* under sulfide exposure

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**Abstract**. Hydrogen sulfide, a common occurrence in hydroelectric dam environment, is potentially toxic to living organisms in the environment. However, studies on the response of indigenous fish species when exposed to sulfide are still lacking in literature. Thus, behavioral responses and mortality of an indigenous fish species, *Barbonymus schwanenfeldii,* were determined in the laboratory where juveniles were exposed to different concentrations of sulfide. Three experiments were conducted during the exposure, namely, gradual sulfide exposure, sulfide exposure under lowering DO condition and gradual sulfide exposure under lowering pH condition. Methylene blue method was used to analyze the water for actual total sulfide concentrations. Behavioral responses, dissolved oxygen level and pH were taken and recorded. Four behavioral responses observed in sulfidic water were huddling, aquatic surface respiration, loss of equilibrium and mortality. However, in negative control no such responses were observed during the duration of the experiment. As the sulfide concentration increased, the time taken to the occurrence of behavioral responses decreased indicating increased toxicity. The $LC_{50}$ at 6 h was estimated as 507.8 µg $L^{-1}$ total sulfide (490 µg $L^{-1}$ $H_2S$) at 95% confidence level. Under sulfide exposure in lowering pH condition, mortality was significantly faster than the other two experimental conditions. This study shows that juveniles showed behavioral response to all sulfide concentrations tested and lowering both DO and pH levels increased the sulfidic toxicity as they led to faster mortality of juveniles.
**Key Words**: huddling, aquatic surface respiration, loss of equilibrium, toxicity, mortality.

**Introduction**. Sulfide is a known naturally produced substance which if in high concentrations can lead to adverse effects to the living organisms and the natural habitat (Bagarinao 1992; Reiffenstein et al 1992; Morii et al 2010). As sulfide is present in low concentrations in the atmosphere, most living organisms are able to withstand it to some extent (Tobler et al 2011). However, few are able to withstand continuous exposure to higher concentration which can be found in aquatic habitat with occasional sulfide concentration peak (Luther III et al 2004; Tobler et al 2011). Sulfide is also associated with deeper column of dam reservoir water in tropical region (Ling et al 2012) and tailwater of hydroelectric dams in temperate region (Nix 1986) and in tropical region (Ling et al 2016). In spite of that, little information is available on the response of an indigenious fish species *Barbonymus schwanenfeldii* when exposed to sulfide. Bagarinao & Lantin-Olaguer (1998) reported the responses of *Chanos chanos* and *Oreochromis* sp. when gradually exposed to hydrogen sulfide in water.

The toxicity of sulfide to aquatic organisms has been linked with the low levels of dissolved oxygen in water. Several studies reported behavioral responses which are similar to behaviors and adaptations under hypoxic waters (Tobler et al 2006; Tobler et al 2011). Behavior is defined as a visible reaction of a living organism to stimuli from a variety of biotic and abiotic factors (Gerhardt 2007). Such responses act as a stress assessment in animals which allow them to evade dangerous stimulus or as a biological characteristic caused by stress (Beitinger 1990) and were often used as early detection of water quality disruption or diseases in fishes. Fishes are often used in models to detect

the presence of contaminants in aquatic habitats due to their sensitivity and abundance (Silva et al 2016). Besides that, sulfide toxicity along with anoxia also led to fishkills in dead-end canals of Delaware Inland Bays (Luther III et al 2004) and it was concluded that the low level of oxygen exaggerated the mortality of fishes in sulfidic condition.

*B. schwanenfeldii*, also known locally as tengadak is an indigenous freshwater species which can be found inhabiting lakes and rivers (Froese & Pauly 2015). The species is said to be distributed widely in Asia including Borneo, Sumatra and Peninsular Malaysia and acts as an important protein source (Mat Isa et al 2012; Froese & Pauly 2015). Changes in water quality parameters such as hydrogen sulfide levels is a rising concern as sulfide affects the survival of fishes in the water column, especially in lentic waters (Tobler et al 2006; Riesch et al 2010; Tobler et al 2011; Henny & Nomosatryo 2012). This could affect the diversity and composition of indigenous fishes such as *B. schwanenfeldii*. Previous studies in a dam and below a dam showed the impacts of dam environment on indigenous fish (Winemiller et al 2016; Nyanti et al 2018a). As three hydroelectric dams have been built in Malaysia and more are being planned, the response of indigenous fish to changes in the environment such as an increase in sulfide requires investigation. Thus, the objectives of this study were to observe the behavioral responses and to determine the mortality of juvenile *B. schwanenfeldii* under different concentrations of hydrogen sulfide exposure.

## Material and Method

***Fish acclimatization***. A total of 600 *B. schwanenfeldii* juveniles were collected from the Inland Fishery Branch of the Department of Agriculture in Tarat of Serian district for the positive control and sulfide exposure experiments. The samples were then acclimatized by adding 25% of the water volume in the bag holding the samples with water from the holding tanks. It was left to acclimatize for 30 minutes and then transferred to the holding tanks. The juveniles were left for a week prior to experiment to prevent stress and fed twice a week. A total of thirty juveniles were weighed by a weighing balance (AND, GH-252).

***Experimental setup***. The experiments were carried out in a modified flow-through bioassay design (Bagarinao & Lantin-Olaguer 1998). The modified design included 15 L airtight containers where the samples were kept during the experiment and aerated freshwater reservoir along with sulfide stock solution. Aerated freshwater and sulfide solution were supplied into the containers at 100 and 5 mL $min^{-1}$, respectively (Figure 1). Both supplies were elevated higher than the containers to allow the flow by gravity and flow rate was controlled using a control valve. Calibrations of flow rates were carried out prior the beginning of each experiment. The temperature was kept at a range of 26-28°C. All experiments including negative control were carried out for 12 h or until all fish died, whichever came first.

***Positive control***. A positive control was carried out to test the suitable sulfide stock solution concentrations and time period of the experiment. The control was run by using the same modified flow-through design with 10 juveniles in each container but with different sets of concentrations. Based on the numerous trials, six different concentrations of sulfide stock solutions were determined and the time periods of the experiments were decided at 12 hours.

***Sulfide stock solution preparation***. For each experiment, six different concentrations of stock solution (6.75, 13.50, 20.25, 27.00, 33.75, 40.50 µg $L^{-1}$) with three replicates were tested on the juveniles and were freshly prepared before the experiment. $Na_2S.9H_2O$ crystals were first thoroughly washed and dried, and then dissolved in 2 L nitrogen-bubbled water to make sulfide stock solution of three concentrations. Nitrogen-bubbled water was prepared by flowing nitrogen gas through water for about 30 minutes in order to reduce the dissolved oxygen level to 1-2 mg $L^{-1}$. The stock sulfide solution bottle was supplied with nitrogen gas to reduce the oxidation of sulfide. For each

concentration, three containers were prepared as replicates. Each container was filled with 12 L aerated freshwater and stocked with ten juveniles.

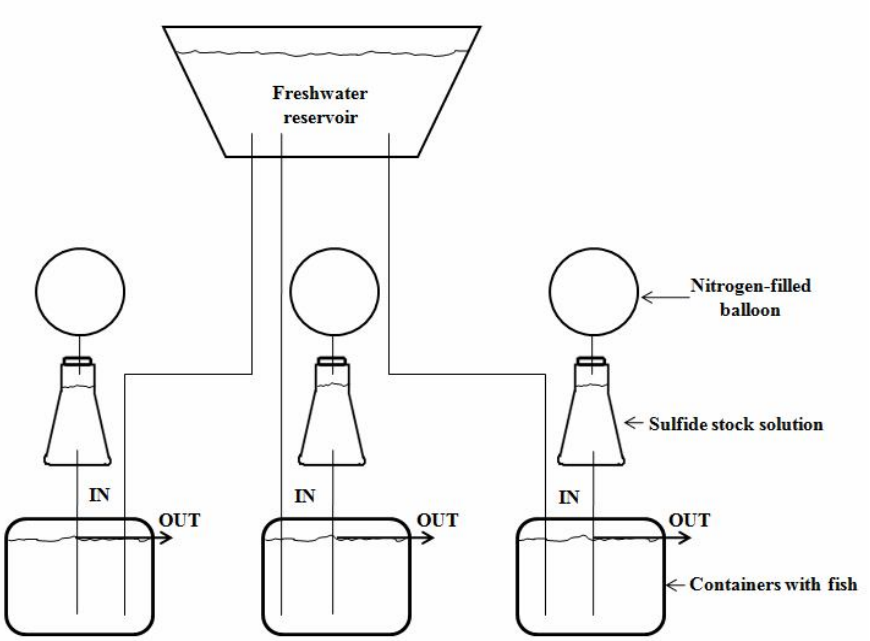


Figure 1. The figure illustrates the flow-through design for sulfide exposure test. For each tested sulfide stock concentration, three sulfide stock solutions equipped with nitrogen-filled balloons were supplied each to the three containers.

***Experiment 1**: **gradual sulfide exposure**.* Gradual sulfide exposure experiment was carried out from August-September 2015 with the juveniles weighing 1.28±0.41 g. This experiment started with aerated water flowing in at the rate of 100 mL $min^{-1}$ and sulfide flowing in at the rate of 5 mL $min^{-1}$ into the containers. Both DO and pH were recorded at 1-hour interval until the end of experiment or until all juveniles died, whichever came first.

***Experiment 2**: **gradual sulfide exposure under lowering DO**.* This experiment was carried out in January 2016 with juveniles weighing 1.06±0.07 g. In this experiment, sulfide was gradually supplied into containers just as in gradual sulfide exposure experiment but without the supply of aerated freshwater or aeration to allow the lowering of DO levels. Before the experiment began, aeration and aerated freshwater (100 mL $min^{-1}$) were supplied into containers stocked with juveniles for a period of 12 hours. When the experiment began, both aeration and freshwater were cut off and sulfide solution started flowing into the containers at 5 mL $min^{-1}$. DO and pH levels were recorded at 1-hour interval and the time of death was also recorded until the end of the experiment. For the negative control, no sulfide was flowed into the containers.

***Experiment 3**: **gradual sulfide exposure under lowering pH**.* This experiment was carried out in February 2016 with juveniles weighing 1.11±0.07 g. During the experiment, aerated freshwater, 10% sulfuric acid, and sulfide were flowing in at 100, 5, and 5 mL $min^{-1}$ respectively into each of the containers. Due to the fast mortality of juveniles in this experiment, DO and pH were recorded at half an hour interval until all fishes died. Negative control was carried out with aerated freshwater and sulfuric acid but without the supply of sulfide.

***Behavioral responses**.* For all the three sulfide exposure experiments, four behavioral responses namely, huddling together, aquatic surface respiration (ASR), loss of equilibrium and turn upside down were monitored and recorded hourly from the start until the end of the experiments.

***Water sampling**.* Based on the findings of positive control, it was decided that the time period of the experiment was 12 hours. The taking of water samples was decided to be at

the 6th hour after the start of the experiment for experiments 1 and 2. However, for experiment 3, water sampling was done at the 3rd hour of experiment period due to its faster mortality response.

***Sample analyses***. The analyses of actual sulfide concentrations from water samples were done following Methylene blue method as provided in the manufacturer's instruction manual for DR2800 (HACH, 2014) and the absorbance was determined by using a spectrophotometer (HACH, DR 2800). Dissolved oxygen and pH levels were taken by using a EXTECH DO Meter/Datalogger SDL150 and a EXTECH pH/ORP Meter SDL100v, respectively.

***Statistical analysis***. To compare the means of time taken to 50% and 100% mortality and total sulfide concentrations between experimental sets, paired *t*-test and one-way ANOVA were used respectively. $LC_{50}$ at 6 h of exposure was plotted at 95% confidence limit. For this study, statistical analyses were carried out by using SPSS version 23.

## Results

***Behavioral responses***. For all the three sulfide exposure experiments, four behavioral responses namely, huddling together, aquatic surface respiration (ASR), loss of equilibrium and turn upside down observed are shown in Table 1. Negative control did not display any response throughout the experimental period of 12 h. In the gradual sulfide exposure, juveniles only displayed huddling together when exposed to 160-196 µg $L^{-1}$ sulfide concentrations. However, all behavioral responses were observed in the other exposure experiments. Based on the results obtained, the responses were sighted occurring earlier when sulfide concentrations were higher.

Table 1

Time taken from the beginning of the experiment to the occurrence of a behavioral response of *Barbonymus schwanenfeldii* in sulfide exposure experiments

| *Sulfide stock solution (µg $L^{-1}$)* | *Container total sulfide (µg $L^{-1}$)* | *Time to behavioral responses (h)* | | | |
|---|---|---|---|---|---|
| | | *Huddle together* | *ASR* | *Loss of equilibrium* | *Turn upside down* |
| *Gradual sulfide exposure* | | | | | |
| 0 | $0^{a}$ | n.d. | n.d. | n.d. | n.d. |
| 6.75 | $160\pm27^{bcd}$ | 8 | n.d. | n.d. | n.d |
| 13.50 | $196\pm35^{cd}$ | 5 | n.d. | n.d. | n.d |
| 20.25 | $350\pm52^{e}$ | 4 | 6 | 7 | 7 |
| 27.00 | $474\pm35^{d}$ | 3 | 5 | 6 | 7 |
| 33.75 | $548\pm46^{f}$ | 2 | 5 | 6 | 6 |
| 40.50 | $659\pm39^{gh}$ | 2 | 5 | 5 | 5 |
| *Sulfide exposure under lowering DO* | | | | | |
| 0 | $0^{a}$ | n.d. | n.d. | n.d. | n.d. |
| 6.75 | $203\pm38^{cd}$ | 7 | 8 | 9 | 10 |
| 13.50 | $340\pm28^{e}$ | 5 | 5 | 7 | 8 |
| 20.25 | $540\pm31^{f}$ | 3 | 4 | 6 | 6 |
| 27.00 | $641\pm22^{g}$ | 2 | 3 | 4 | 6 |
| 33.75 | $734\pm35^{h}$ | 2 | 2 | 3 | 4 |
| 40.50 | $946\pm45^{i}$ | 1 | 2 | 3 | 4 |
| *Sulfide exposure under lowering pH* | | | | | |
| 0 | $0^{a}$ | n.d. | n.d. | n.d. | n.d. |
| 6.75 | $76\pm10^{ab}$ | 3 | 3.5 | 5.5 | 6.5 |
| 13.50 | $127\pm5^{bc}$ | 2.5 | 3 | 3.5 | 4 |
| 20.25 | $166\pm22^{bcd}$ | 2 | 2.5 | 3 | 4 |
| 27.00 | $208\pm36^{cd}$ | 1.5 | 2 | 2 | 2 |
| 33.75 | $233\pm7^{d}$ | 1 | 1.5 | 2 | 2 |
| 40.50 | $326\pm5^{e}$ | 0.5 | 1 | 1.5 | 2 |

*n.d. = not detected during the 12-hour experimental duration; ** the same superscript indicates no significant difference ($p > 0.05$) between container total sulfide concentrations; **total of juveniles for each tested sulfide concentration was 10, meaning N=190 for each sulfide exposure experiment.

***Gradual sulfide exposure***. In gradual sulfide exposure experiment, where temperature was 26-28°C, 100% mortality of juveniles was recorded the earliest at 6 h with actual total sulfide concentration of 659 µg $L^{-1}$ (Table 2). $LC_{50}$ at 6 h was estimated by probit regression as 507.8 µg $L^{-1}$ total sulfide (490 µg $L^{-1}$ $H_2S$) at 95% confidence level (Figure 2). During the course of the experiment, DO level declined from 5.85±0.05 mg $L^{-1}$ at the start to 2.78±0.23 mg $L^{-1}$ at the end while pH at the start was 7.00±0.11 and 5.54±0.10 at the end. For the negative control, at the end of the experiment, DO and pH were 2.55±0.21 mg $L^{-1}$ and 5.57±0.02, respectively. The DO and pH levels of negative control and sulfide exposure declined to a range of 2.55-2.78 mg $L^{-1}$ and 5.57-5.54, respectively. However, mortality was only observed in sulfide exposure experiment which indicates that the mortality was due to sulfide introduced in the water.

Table 2

Time to mortality of juveniles *Barbonymus schwanenfeldii* exposed to different sulfide levels and negative control

| *Sulfide stock solution (µg $L^{-1}$)* | *Container total sulfide (µg $L^{-1}$)* | *Time to mortality (h)*** | |
|---|---|---|---|
| | | *50 %* | *100 %* |
| 0* | 0* | > 12 | > 12 |
| 6.75 | 160 | > 12 | > 12 |
| 13.50 | 196 | > 12 | > 12 |
| 20.25 | 350 | 10±1 | 11±1 |
| 27.00 | 474 | 7±1 | 9±0 |
| 33.75 | 548 | 6±1 | 7±1 |
| 40.50 | 659 | 5±0 | 6±0 |

*Negative control, no sulfide was supplied to this set; **Time to 50% and 100% mortality are significantly different (paired *t*-test, p > 0.05).

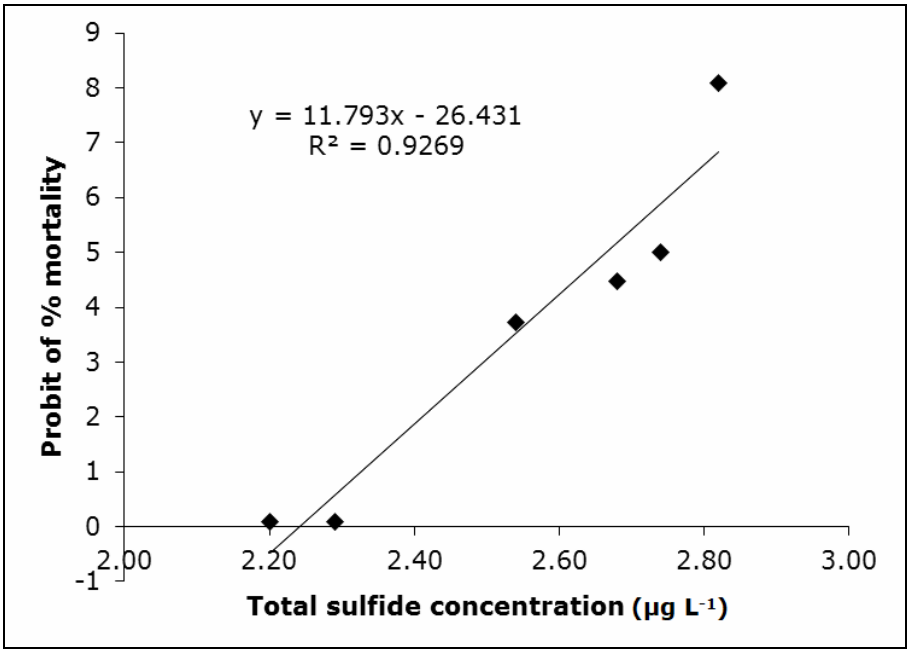


Figure 2. Probit regression of percentage of mortality against log of total sulfide concentration to obtain $LC_{50}$ at 6 h for *Barbonymus schwanenfeldii.*

***Sulfide exposure under lowering DO***. For the negative control, no mortality was recorded throughout the 12 h period with similar DO and pH declines from 5.90 to 2.50 mg $L^{-1}$ and from 7.35 to 5.78 respectively during the course of the experiment (Figure 3).

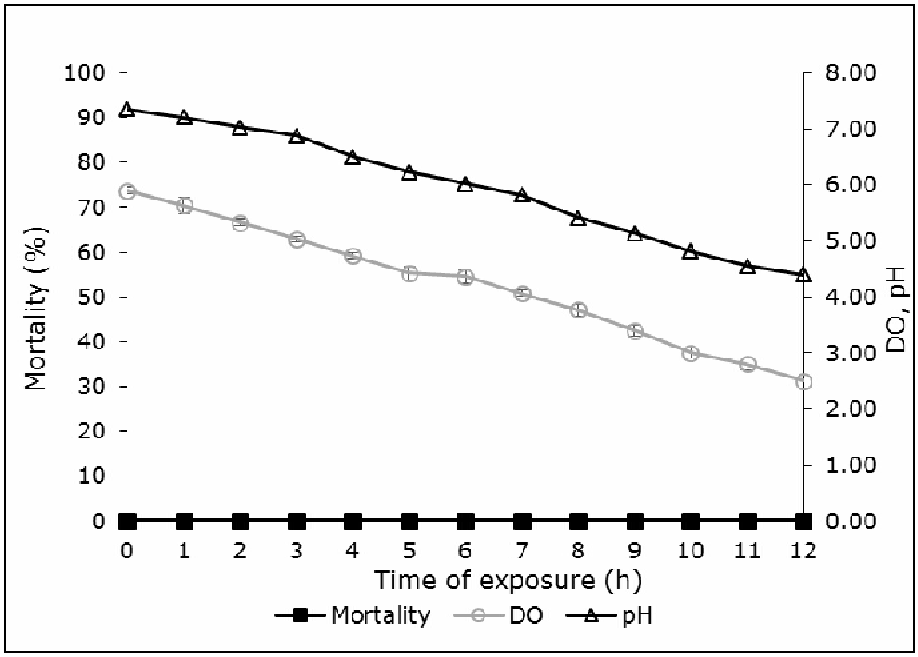

Figure 3. The mortality of juveniles under lowering DO in negative control.

Figure 4 shows line graphs depicting the pattern of mortality against the time of sulfide exposure and the comparisons between mortality in gradual sulfide exposure and sulfide exposure under lowering DO. The trend shows that the mortality rate was maintained at zero percent for a few hours before a sudden increase in mortality until it reached 100%. In both of the exposure experiments, 100% mortality was observed within 3-4 hours after the first occurrence of death in all concentrations. By comparing the mortality trends between the experiments when container sulfide concentrations were not significantly different, no apparent differences were observed. 100% mortality was reached at the same hour for 350 and 340 µg $L^{-1}$, whereas an hour later for sulfide exposure under lowering DO (540 and 641 µg $L^{-1}$) than gradual sulfide exposure (548 and 659 µg $L^{-1}$).

***Gradual sulfide exposure under lowering pH***. The negative control DO value decreased from 7.00 to 2.83 mg $L^{-1}$ while the pH levels declined from 7.64 to 3.23 during the experimental duration (Figure 5). The first occurrence of mortality was at 2.5$^{th}$ hour (pH 5.62, DO at 3.43 mg $L^{-1}$) and 100% mortality was reached at 5.5 h (pH 3.23, DO at 2.83 mg $L^{-1}$).

The supply of sulfuric acid ensured gradual decline of pH which led to faster mortality (Figure 6). The juveniles reached 100% mortality earliest at the 3$^{rd}$ hour (208 µg $L^{-1}$) under sulfide exposure with lowering pH whereas when exposed to sulfide only, no mortality (196 µg $L^{-1}$) was recorded under 12 h period. This shows that lowering pH aggravated the mortality of *B. schwanenfeldii* juveniles due to sulfide toxicity. Under the influence of lowering pH, juveniles in 326 µg $L^{-1}$ reached 100% mortality (3$^{rd}$ hour) faster compared to sulfide concentration of 350 µg $L^{-1}$ in sulfide exposure only (11$^{th}$ hour).

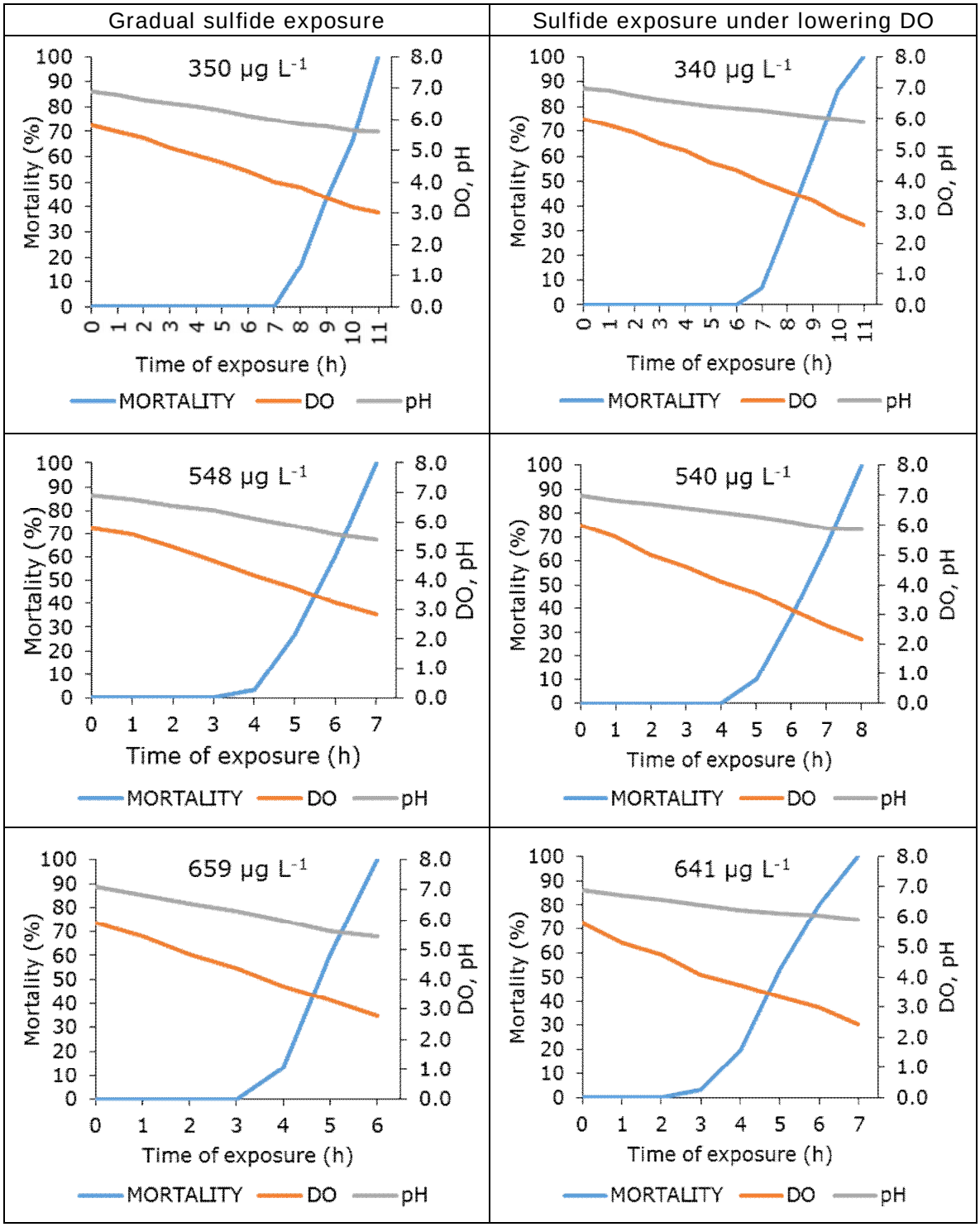


Figure 4. Mortality of *Barbonymus schwanenfeldii* during gradual sulfide only (350, 548 and 659 µg $L^{-1}$) and sulfide exposure under lowering DO (340, 540 and 641 µg $L^{-1}$).

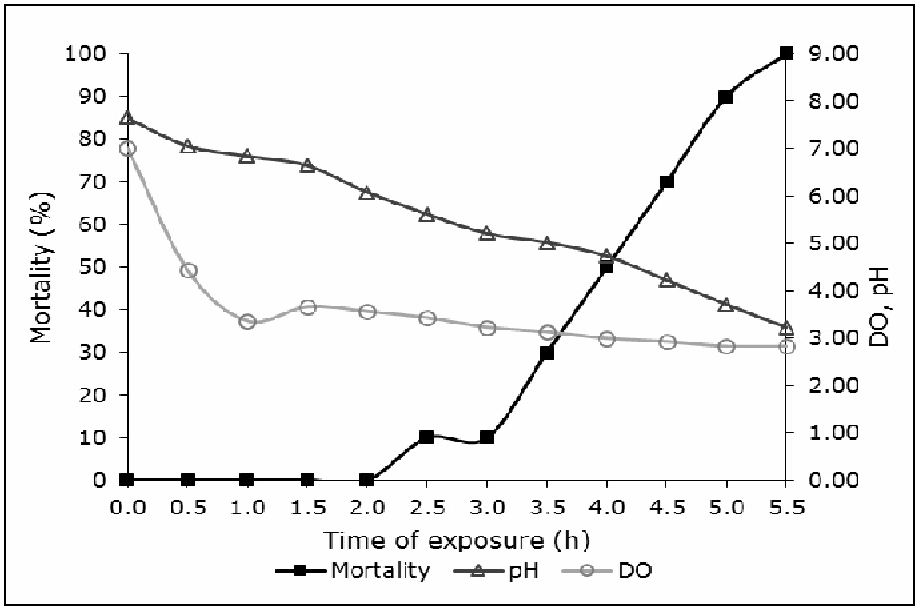


Figure 5. The mortality of juveniles under lowering pH in negative control.

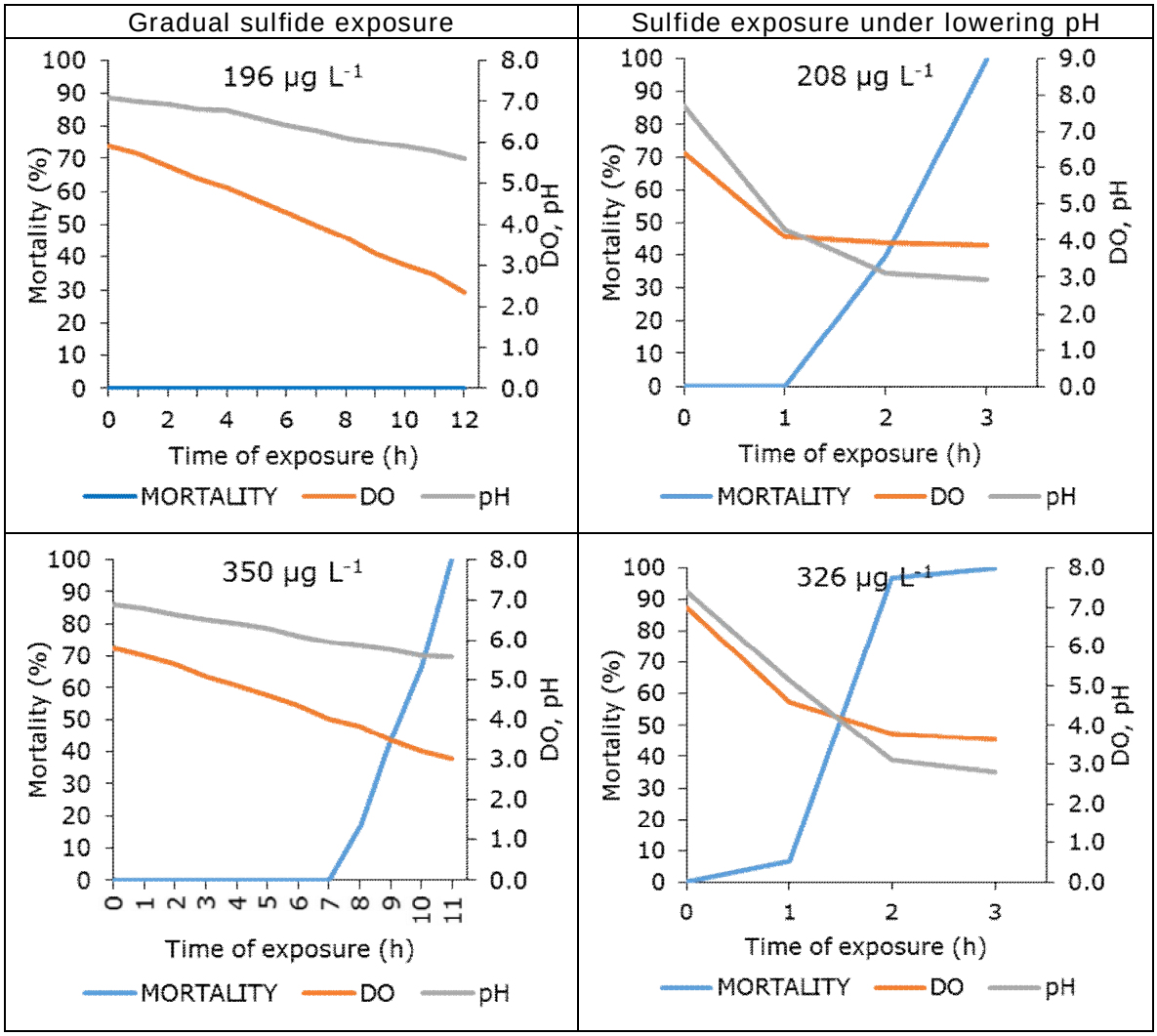


Figure 6. Mortality of *Barbonymus schwanenfeldii* during gradual sulfide only (196 and 350 µg $L^{-1}$) and gradual sulfide under lowering pH (208 and 326 µg $L^{-1}$).

***Sulfide exposure under lowering DO and pH***. Following the results obtained from sulfide exposure under lowering DO and pH, the mortality against time of exposure were compared. Figure 7 showed that under lowering pH concentrations, mortality was reached faster than in lowering DO even with close value of sulfide concentrations. The end DO levels in lowering pH experiment declined drastically with pH unlike in the experiment of lowering DO. It can be observed that under when both DO and pH levels dropped drastically with the increase of sulfide, the mortality was faster.

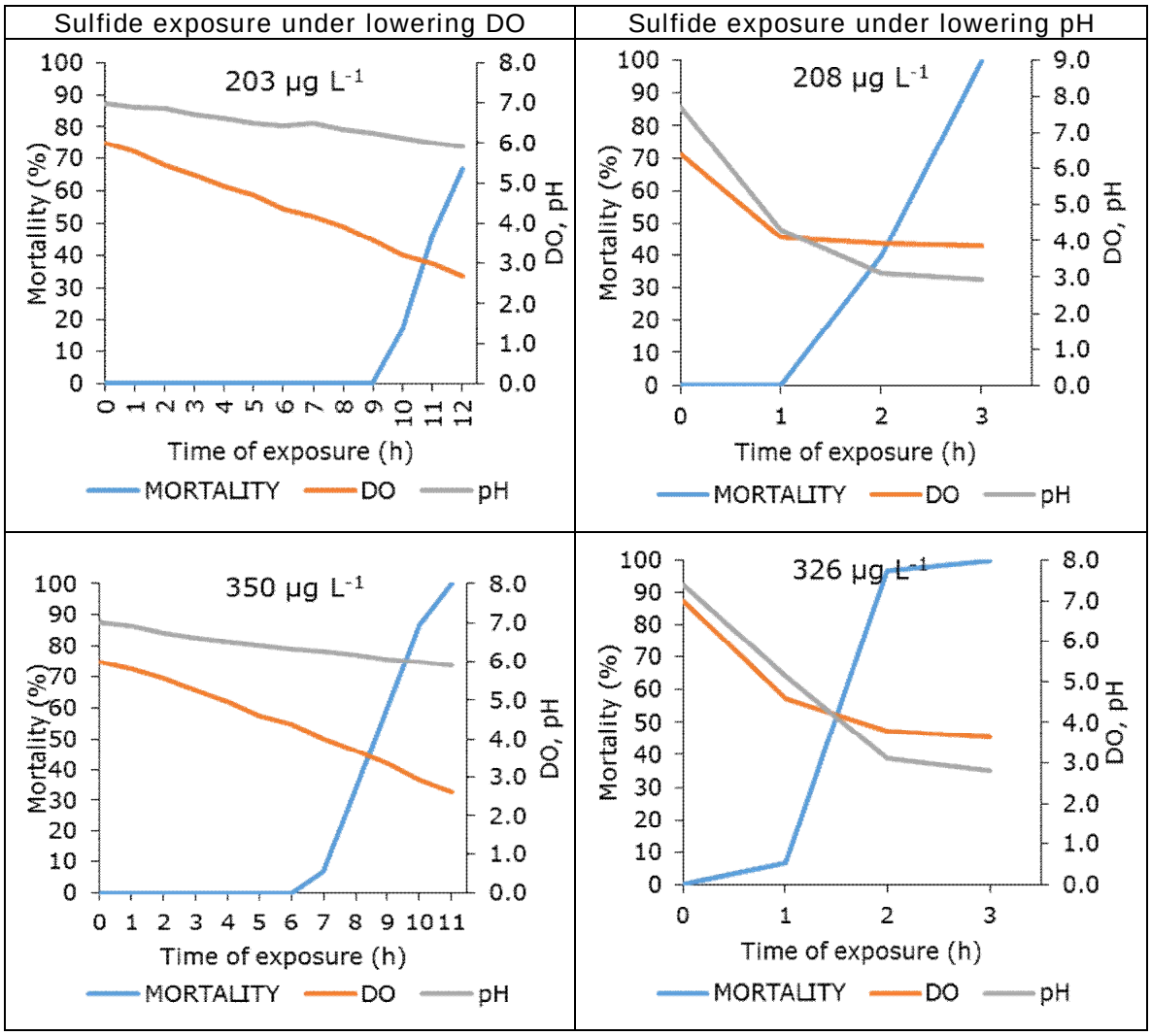


Figure 7. Mortality of *Barbonymus schwanenfeldii* during sulfide exposure under lowering DO (203 and 350 µg $L^{-1}$) and sulfide exposure under lowering pH (208 and 326 µg $L^{-1}$).

**Discussion**. Behavioral responses due to contaminant exposure are said to be an endpoint to sub-lethal toxicity (Calfee et al 2016). In this study, four behavioral responses were visually observed on the juveniles of *B. schwanenfeldii* upon the exposure to sulfide stock solution ranging from 6.75 to 40.50 µg $L^{-1}$. Huddling together of the juveniles were the earliest response displayed. Such behavior was categorized as fright behavior displayed by fish when reacting to the gradual introduction of sulfide into the water (Bisson & Bilby 1982). The juveniles appeared to move rapidly in huddling form. Mason & Chapman (1965) observed such movements in juvenile coho salmon in experimental stream systems and speculated it was due to the sudden changes in the water. Thus, huddling together is a form of fright response towards apparent changes in water. Aquatic surface respiration is a form of air breathing in which the fishes came up to the air-water interface to 'gasp' for air (Plath et al 2007a) as the interaction of air with the water surface allows diffusion of oxygen. A study by Affonso et al (2002) reported that in addition to air breathing, *Colossoma macropomum* swells its lower lip in order to

direct oxygenated water at the surface through its mouth passing the gills. The behavior shown by *C. macropomum* was one of the forms of respiratory adaptation towards temporary fluctuation of sulfide in the water. Apart from that, other adaptions such as enlargement of gill surface and ability to switch to anaerobic metabolism were displayed by some fish species under the influence of temporary sulfidic environment to allow efficient oxygen uptake (Affonso et al 2002; Plath et al 2007b). For fishes that regularly experience sulfide fluctuation, the adaptation does not take up as much as energy as those in species that are not of sulfidic environment.

Swimming pattern of the juveniles was also affected after quite some time from the start of the exposure. The juveniles were seen to loss equilibrium and swam around while spinning around in a rapid movement. According to Calfee et al (2016), changes in swimming behavior is a valid index of sub lethal indication in toxicity tests. Based on the observation during the experiment, once the juveniles displayed loss of equilibrium, it only took one hour or less before the death of the juveniles. Apart from the observed behavioral patterns in this study, sulfide was also reported to affect feeding activity in teleost fishes (Mahavadiya et al 2018).

Prior to the start of all sulfide exposure experiment, a set of trials were done to determine the best concentrations of sulfide stock solutions and the experimental design. In both Experiment 1 and Experiment 2, water samples were taken at the $6^{th}$ hour for total sulfide analyses. This is because the trial showed that the earliest possible 100% mortality was at the $6^{th}$ hour. Due to this reason, the sampling time was set at the $6^{th}$ hour of the experimental period.

Sulfide tolerance is also affected by the body mass of the organisms. The mean weight of the juveniles in the gradual sulfide experiment was relatively small, 1.28±041 g. According to a study done by Tobler et al (2011), smaller sized fish are able to withstand higher sulfide concentrations compared to large sized fish. However, in the study of sulfide tolerance on milkfish and tilapia by Bagarinao & Lantin-Olaguer (1998), smaller sized fish was unable to tolerate high sulfide concentrations than larger sized fish. It could be assumed that the tolerance of fish to sulfide in relation to body mass is also dependent on the species itself.

At the beginning of sulfide exposure with low DO, aeration and aerated freshwater supply were turned off to allow gradual decline of DO throughout the experiment. A study done on the tolerance of *B. schwanenfeldii* in prolonged low DO levels reported that the species reached zero survival at DO levels 0.0 and 2.0 mg $L^{-1}$ (Nyanti et al 2018b). However, in this study, the species did not die under lowering DO levels to ~2.0 mg $L^{-1}$ in the negative control. This could be due to the period of exposure of the juveniles in these two studies in which Nyanti et al (2018b) exposed the species in prolonged low DO environment and this study only exposed the juveniles in 12 h. The severity of sulfide toxicity was exaggerated as the dissolved oxygen levels decreased (Bagarinao & Lantin-Olaguer 1998). This was also reported in other studies in natural habitat such as Kump et al (2005), Plath et al (2007a, b) and Riesch et al (2010). It was reported that DO was usually found to be low in higher sulfide concentration areas. In the natural habitat, the lack of oxygen during hypoxic or anoxic state to oxidize organic matter leads to sulfate reduction (Vaquer-Sunyer & Duarte 2010). Under such condition, sulfide concentrations were elevated in the water column. As mentioned before, most adaptation by fishes to sulfide was mostly the same as to adaptation to hypoxic waters. Prolonged exposure to hypoxic or anoxic condition caused aquatic species to be more susceptible to diseases and even mortalities (Wannamaker & Rice 2000; Luther III et al 2004). According to a study in dead-end canals by Luther III et al (2004), the most common cause of anoxia and stratification were eutrophication due to high temperature and nutrient concentrations. Eutrophication leads to an increase of organic matter which is decomposed by sulfate reducing bacteria, producing $H_2S$. This shows that high sulfide levels and low DO caused and aggravated mortalities as observed in the present experiment.

In the gradual sulfide exposure under lowing pH, sulfuric acid was supplied to the containers to ensure gradual decrease of pH. Based on the earlier sulfide exposure experiments done, exposure under low DO lead to slightly earlier mortality than exposure

to sulfide only. The result showed faster mortality in low pH sulfidic water (166-326 µg $L^{-1}$) compared to sulfidic water only (196-350 µg $L^{-1}$). Nyanti et al (2017) reported that at pH 4.5, *B. schwanenfeldii* died on the first day of exposure. This supports the negative control in this study in which the mortality of the species was continuous once it reached pH ~4.5. Lower pH and sulfidic condition was reported to cause mortality and reduced diversity of fishes (Tobler et al 2006; Richards & Pallud 2016). The supply of hydrogen sulfide, a weak acid (Hughes et al 2009) and sulfuric acid into the tank lowered the pH of the waters which is observed in the experiments. The lowering pH levels intensified the toxicity of sulfide due to the increase of $H_2S$ in water based on its pH dependent distribution characteristic. Toxic $H_2S$ is dominant in water with pH below 7 and $HS^-$ is dominant in pH higher than 7 (APHA 2005; Hughes et al 2009), which leads to higher concentrations of $H_2S$. This condition allows the sulfide toxicity to be stronger under acidic or low pH levels as the hazardous $H_2S$ are dominant.

**Conclusions**. Juveniles *Barbonymus schwanenfeldii* showed behavioral responses upon the gradual sulfide exposure at as low a concentration as 160 µg $L^{-1}$ due to toxicity which is not observed in negative control. Huddling, aquatic surface respiration, and loss of equilibrium were observed during the study. Such responses were an act of adaptations to the sulfidic environment. However, prolonged exposure led to mortality of the juveniles. As sulfide concentration increased, the time taken to the first observation of a behavioral response and also juvenile mortality decreased. The toxicity of sulfide was aggravated under low oxygen and pH levels.

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## References

Affonso E. G., Polez V. L. P., Correa C. F., Mazon A. F., Araujo M. R. R., Moraes G., Rantin F. T., 2002 Blood parameters and metabolites in the teleost fish *Colossoma macropomum* exposed to sulfide or hypoxia. Comparative Biochemistry and Physiology Part C 133:375-382.

American Public Health Association (APHA), 2005 Standard methods for the examination of water and wastewater. 21st edition, American Public Health Association, Washington, 1220 pp.

Bagarinao T., 1992 Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquatic Toxicology 24:21-62.

Bagarinao T., Lantin-Olaguer I., 1998 The sulfide tolerance of milkfish and tilapia in relation to fish kills in farms and natural waters in the Philippines. Hydrobiologia 382:137-150.

Beitinger T. L., 1990 Behavioral reactions for the assessment of stress in fishes. Journal of Great Lakes Research 16(4):495-528.

Bisson P. A., Bilby R. E., 1982 Avoidance of suspended sediment by juvenile coho salmon. North American Journal of Fisheries Management 4:371-374.

Calfee R. D., Puglis H. J., Little E. E., Brumbaugh W. G., Mebane C. A., 2016 Quantifying fish swimming behavior in response to acute exposure of aqueous copper using computer assisted video and digital image analysis. Journal of Visualized Experiments 108:53477.

Froese R., Pauly D., 2015 Fishbase. Available at: http://www.fishbase.org. Accessed: February, 2015.

Gerhardt A., 2007 Aquatic behavioral ecotoxicology - prospects and limitations. Human and Ecological Risk Assessment 13(1-3):481-491.

Henny C., Nomosatryo S., 2012 [Sulfide dynamic in Danau Manijau: implications on phosphate release in hypolimnion]. Prosiding Seminar Nasional Limnologi VI, 2010:91-106. [in Indonesian]

Hughes M. N., Cantelles M. N., Moore K. P., 2009 Making and working with hydrogen sulfide: the chemistry and generation of hydrogen sulfide *in vitro* and its measurement *in vivo*: a review. Free Radical Biology and Medicine 47:1346-1353.
Kump L. R., Pavlov A., Arthur M. A., 2005 Massive release of hydrogen sulfide to the surface ocean and atmosphere during intervals of oceanic anoxia. Geology 33:397-400.
Ling T. Y., Paka D. D., Nyanti L., Ismail N., Emang J. J. J., 2012 Water quality at Batang Ai hydroelectric reservoir (Sarawak, Malaysia) and implications for aquaculture. International Journal of Applied Science and Technology 2(6):23-30.
Ling T. Y., Soo C. L., Heng T. L. E., Nyanti L., Sim S. F., Grinang J., 2016 Physicochemical characteristics of river water downstream of a large tropical hydroelectric dam. Journal of Chemistry 2016:1-7.
Luther III G. W., Ma S., Trouwborst R., Glazer B., Blickley M., Scarborough R. W., Mensinger M. G., 2004 The roles of anoxia, $H_2S$, and storm events in fish kills of dead-end canals of Delaware inland bays. Estuaries 27:551-560.
Mahavadiya D., Sapra D., Rathod V., Sarman V., 2018 Effect of biotic and abiotic factors in feeding activity in teleost fish: a review. Journal of Entomology and Zoology Studies 6(3):387-390.
Mason J. C., Chapman D. W., 1965 Significance of early emergence, environmental rearing capacity, and behavioral ecology of juvenile coho salmon in stream channels. Journal of the Fisheries Research Board of Canada 22:173-190.
Mat Isa M., Md-Shah A. S., Mohd-Sah S. A., Baharudin N., Abdul-Halim M. A., 2012 Population dynamics of tinfoil barb, *Barbonymus schwanenfeldii* (Bleeker, 1853) in Pedu Reservoir, Kedah. Journal of Biology, Agriculture and Healthcare 2(5):55-69.
Morii D., Miyagatani Y., Nakamae N., Murao M., Taniyama K., 2010 Japanese experience of hydrogen sulfide: the suicide craze in 2008. Journal of Occupational Medicine and Toxicology 5:1-3.
Nix J., 1986 Spatial and temporal distribution of sulfide and reduced metals in the tailwater of Narrows dam (Lake Greeson), Arkansas. Technical Report E-86-14, US Army Corps of Engineers, DC, 103 pp.
Nyanti L., Abu-Rashid N. N. K., Ling T. Y., Soo C. L., Sim S. F., Grinang J., 2017 Acidification tolerance of *Barbonymus schwanenfeldii* (Bleeker, 1854) and *Oreochromis niloticus* (Linnaeus, 1758) - implication of fish size. AACL Bioflux 10(4):746-753.
Nyanti L., Noor-Azhar N. I., Soo C. L., Ling T. Y., Sim S. F., Grinang J., Ganyai T., Lee K. S. P., 2018a Physicochemical parameters and fish assemblages in the downstream river of a tropical hydroelectric dam subjected to diurnal changes in flow. International Journal of Ecology 2018:article ID 8690948, 9 pp.
Nyanti L., Soo C. L., Ahmad-Tarmizi N. N., Abu-Rashid N. N. K., Ling T. Y., Sim S. F., Grinang J., Ganyai T., Lee K. S. P., 2018b Effects of water temperature, dissolved oxygen and total suspended solids on juvenile *Barbonymus schwanenfeldii* (Bleeker, 1854) and *Oreochromis niloticus* (Linnaeus, 1758). AACL Bioflux 11(2):394-406.
Plath M., Tobler M., Riesch R., Garcia de Leon F. J., Giere O., Schlupp I., 2007a Survival in an extreme habitat: the roles of behaviour and energy limitation. Naturwissenschaften 94:991-996.
Plath M., Hauswaldt J. S., Moll K., Tobler M., Garcia de Leon F. J., Schlupp I., Tiedemann R., 2007b Local adaptation and pronounced genetic differentiation in an extremophile fish, *Poecilia mexicana*, inhabiting a Mexican cave with toxic hydrogen sulphide. Molecular Ecology 16:967-976.
Reiffenstein R. J., Hulbert W. C., Roth S. H., 1992 Toxicology of hydrogen sulfide. Annual Review of Pharmacology and Toxicology 32:109-134.
Richards C. M., Pallud C., 2016 Kinetics of sulfate reduction and sulfide precipitation rates in sediments of a bar-built estuary (Pescadero, California). Water Research 94:86-102.
Riesch R., Plath M., Schlupp I., 2010 Toxic hydrogen sulfide and dark caves: life-history adaptations in a livebearing fish (*Poecilia mexicana*, Poeciliidae). Ecology 91:1494-1505.

Silva S. V. S., Dias A. H. C., Dutra E. S., Pavanin A. L., Morelli S., Pereira B. B., 2016 The impact of water pollution on fish species in southeast region of Goiás, Brazil. Journal of Toxicology and Environmental Health, Part A 79(1):8-16.
Tobler M., Schlupp I., Heubel K. U., Riesch R., Garcia de Leon F. J., Giere O., Plath M., 2006 Life on the edge: hydrogen sulfide and the fish communities of a Mexican cave and surrounding waters. Extremophiles 10:577-585.
Tobler M., Palacios M., Chapman L. J., Mitrofanov I., Bierbach D., Plath M., Arias-Rodriguez L., Garcia de León F. J., Mateos M., 2011 Evolution in extreme environments: replicated phenotypic differentiation in live bearing fish inhabiting sulfidic springs. Evolution 65:2213-2228.
Vaquer-Sunyer R., Duarte C. M., 2010 Sulfide exposure accelerates hypoxia-driven mortality. Limnology and Oceanography 55:1075-1082.
Wannamaker C. M., Rice J. A., 2000 Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. Journal of Experimental Marine Biology and Ecology 249:145-163.
Winemiller K. O., McIntyre P. B., Castello L., Fluet-Chouinard E., Giarrizzo T., Nam S., Baird I. G., Darwall W., Lujan N. K., Harrison I., Stiassny M. L. J., Silvano R. A. M., Fitzgerald D. B., Pelicice F. M., Agostinho A. A., Gomes L. C., Albert J. S., Baran E., Petrere Jr. M., Zarfl C., Mulligan M., Sullivan J. P., Arantes C. C., Sousa L. M., Koning A. A., Hoeinǵhaus D. J., Sabaj M., Lundberg J. G., Armbruster J., Thieme M. L., Petry P., Zuanon J., Torrente Vilara G., Snoeks J., Ou C., Rainboth W., Pavanelli C. S., Akama A., van Soesberǵen A., Sáenz L., 2016 Balancing hydropower and biodiversity in the Amazon, Congo and Mekong. Science 351(6269):128-129.

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