

## Effect of Peat Water Mix with Borehole Water on Mortality of African Catfish

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**ABSTRACT** Generally, cultured fish are suitable in media with a pH of around 7, like mountainous river and borehole water. However, peat water is known with low pH. It is not suitable for fish culture, especially for fingerlings. This study was conducted to examine the survival of African catfish fingerling (*Clarias gariepinus*) in the mixture of peat and borehole water with different pHs. Survival test of African catfish as subject applied bioassay method, LC<sub>50</sub>-96 hours. Peat water as the agent was arranged for 11 concentration levels of 0% - 100% with no replication and placed in plastic basins with 20 liters each. Catfish fingerlings were used for subject fish (size 10-15 cm) and 10 individuals in each basin. Water quality, mortality, and behavior of fish were observed. Results showed that the survival ability of African catfish on peat water, the LC<sub>50</sub>-96 hours was 63.8%, with 95% CI between 53.3% and 76.4% in concentration. Behavioral response of fish fingerling exposed to peat water  $\geq$  40% in the beginning 2 hours was frequently swimming up to surface. Lethal responses fingerlings were frequently taking up oxygen to the water surface, horizontally swimming, and moving vertically before death. After death, the fish ran out of mucus, and the skin looked peeling and bruising.

**Keywords:** African catfish fingerlings; bioassay; peat water of low pH; survival

### INTRODUCTION

Peat water is abundant in parts of the islands of Kalimantan and Sumatra, with high acidity and low pH. Visually, peat water looks dark brown. Its organic content in subtropical peatlands (boreal peatland) is predominantly humic acid, as indicated by high DOC (Steinberg, 2003). However, in peat water in the tropics, especially Kalimantan, the high of DOC is dominated by fulvic acid (Ishikawa et al., 2005). According to Ishikawa et al. (2006), in Central Kalimantan rivers and lakes whose water is supplied from peatlands, the DOC content ranges from 5-50 mgC/l. The DOC content is exponentially proportional to decreasing in pH value and increasing in the colour of the peat water (Wetzel et al., 1995; Ishikawa et al., 2006; Ardianor et al., 2012). In many water bodies, rivers, lakes, and swamps in Central Kalimantan affected by peatlands, pH values vary between 3.45 and 5.92 (Ardianor et al., 2012).

According to Husson et al. (2018) and Thornton et al. (2018), peat waters, such as the Sebangau River, naturally live in various aquatic biotas like plankton and aquatic invertebrates, and fish. Local fish, which are generally blackfish, have other respiratory organs that are known to be adaptive to peat waters with low oxygen. However, Thornton et al. (2018) also found the ordinary river fish, which are generally called white fishes, such as the *lais* (Siluridae) and *baung* (Bagridae) groups at Sebangau River. The white fishes may have migrated and adapted to the peat water of Sebangau River since the construction of the canals of the mega rice project, which have connected the Kahayan River and Sebangau Rivers. Likewise, apart from being the living media for aquatic biota, peat waters may also be used as the media for rearing cultured fish with specific techniques or technologies under conditions

of high acidity and low pH.

Cultured fishes have been known to have fast growth and usually eat artificial food (feed pellets), and can develop in the water of pH around 7. However, at the fingerlings stage, they are difficult to survive in peat water with low pH of around 4. The survival test applying bioassay, LC<sub>50</sub>-96 hours of several cultured fish species showed that tilapia (*Oreochromis* sp.) fingerlings, 3-5 cm in length, have been able to survive on only a maximum of 15.8% of peat water with low pH of about 4; and similarly to striped catfish (*Pangasionodon hypophthalmus*) fingerlings, 5-8 cm in length have been at maximum 28.7% of peat water (Ardianor et al., 2012). Likewise, LC<sub>50</sub>-96 hours of giant gourami (*Osphronemus gourami*) fingerlings, 3-5 cm in length, have survived in a maximum of 35% of peat water (Pusparani, 2020).

African catfish has been one of the favourite cultured fish species. So far, its survival in peat water of low pH has not been known with certainty, although Mustapha & Mohammed (2018) studied catfish (the African mud catch fish, *Clarias gariepinus*) in low pH of the ordinary water media. In peat waters of the Sebangau River watershed, local catfish (*C. batrachus*) exist naturally in peat waters of low pH (Thornton et al., 2018). Taxonomic classification and identity of African catfish, *C. gariepinus*, are written by Saanin (1984) and Iswanto (2013), including the morphology by Najiyati (2003) and the behaviour by Khairuman (2010).

This study examines the survival of African catfish (*C. gariepinus*) fingerlings in the mixture of peat water with low pH and borehole water. Results hope to see the prospect of catfish being cultivated in a recirculation system

consisting of a culture tank and additional information for studying the adaptation of a cultured fish in the tank that could be applied to peat waters.

## MATERIALS AND METHODS

### Materials

Apparatus and equipment utilized for this treatment were 11 plastic basins (with the capacity of 35 litres), 6 electric aerators, aerator tube with stone (11 pieces), electrical balance, Erlenmeyer, beaker glass, pH Meter Horiba type D74, DO meter Horiba type OM-12 dan fibre tank (1200 litres). Materials used were peat water of low pH, borehole water (groundwater), and African catfish fingerlings (10-15 cm in length) amount of 110 inds. and slaked lime ( $\text{Ca}(\text{OH})_2$ ).

### Methods

The bioassay test was conducted in a research installation from 22 - 26 December 2021. Activities included mobilizing peat water from the primary canal in *Kalampangan* village, arranging treatment units, releasing the subject fish, and sampling and measuring the water quality parameters.

The use of biological tests or bioassay to observe the survival ability of catfish in peat water of low pH is because this method has been standard in assessing the lethal response of fish as the subject to the toxic substance or substances, such as peat water. According to [Sprague \(1973\)](#), the biological test, toxicity test, or bioassay test is a test in which the levels and workings of a substance are measured by the reaction of an organism to the substance. [Hubert \(1980\)](#) also describes a bioassay as a body of procedure in which the subject's response determines the amount or strength of an agent or stimulus. The subject is usually an animal, a human tissue, or a bacterial culture. The agent is the drug, and the response is a change in a particular characteristic or even the subject's death. [APHA \(1995\)](#) defines a toxicity test or bioassay as a procedure to evaluate the response of aquatic biota in detecting the presence or effect of one or more pollutants, waste, or environmental Physico-chemical factors, alone or in combination. [Baird & Bridgewater's \(2017\)](#) toxicity test is needed to evaluate water quality because chemical and physical tests alone are insufficient to assess potential effects on aquatic biota.

The procedure for carrying out the test, in the beginning, was to prepare 11 plastic basins (experimental unit) with no replication. Basins were previously washed with detergent, rinsed several times, and dried for sterilization. Each basin was filled with a mixture of peat water and borehole water, as strength or agent, until a volume limit of 20 litres, where the percentage of peat water was respectively 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% in every unit treatment basin. The 0% concentration was entirely borehole water, while 100% was ultimately peat water, and the others, such as 10% - 90%, were a mixture of borehole water and peat water which was done in such away. The peat water used for reared media was taken from the primary canal of the former 1 million ha project in *Kalampangan Village*, *Palangka Raya*, with a pH between 3 and 4.

Meanwhile, borehole water was groundwater pumped from

a depth of about 20 m in which its pH ranged from 4.5 to 5.5. This pH was then increased to about 7 with a slaked lime amount of 185 mg per litre. The limed borehole water with 200 litres was stocked in a big tank. Each tested plastic basin was installed with a single point of aeration with an electric aerator for oxygen supply during the 96 hours test period. The fish to be tested (subject) were the African catfish fingerlings with the size of 10 - 15 cm in length (average of 14.2 cm,  $n=10$ ).

The number of subjects (tested fish) on each tank was 10 inds. Each basin measured the initial value of water quality, such as temperature, pH, and EC, using pH Meter Horiba type D74 and DO meter Horiba type OM-12, Japan. For pH, the initial values of the mixture of peat and borehole water, 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% were respectively 6.56, 6.24, 5.93, 4.82, 4.46, 4.26, 3.95, 3.52, 3.34, 2.28, and 3.22. After being stocked, the mortality of the subject was observed in a series of times, 1, 2, 4, 8, 10, 12, 18, 36, 48, 60, 72, 84, and 96 hours, in line with the time observing the behavioural responses and morphological abnormalities according to [Mustapha & Mohammed \(2018\)](#) of fish as well as water quality.

The median lethal concentration,  $LC_{50}$ -96 hours of tested fish on peat water of low pH, was estimated using the Dragsted-Bahren interpolation formula, including a 95% confidence interval ([Hubert, 1980](#)), with the formula:

$$m = \log_{10} D_{lower} + \left[ \left( \frac{50 - Y_{lower}}{Y_{upper} - Y_{lower}} \right) \right]$$

$m$  = Median Lethal Concentration  
 $\log_{10} D_{lower}$  =  $\log_{10}$  dosage/concentration lower median  
 $Y_{lower}$  = Y lower of median estimation  
 $Y_{upper}$  = Y uppermedian estimation

$$SE(m) = \sqrt{\frac{0.79 \cdot (h) \cdot IR}{n}}$$

Where :  $SE(m)$  = standard error ( $m$ )

$$h = \frac{(\text{maxdosage} - \text{mindosage})}{\sum \text{interval}}$$

$$IR = \log LC_{75} - \log LC_{25}$$

Calculation and drawing graphs were done using MS Office excel 2010 and R: A language and environment for statistical computing programs, ver.4.1.1 ([R Core Team, 2021](#)).

## RESULTS AND DISCUSSION

Evaluation of survival of African catfish to peat water media of low pH is needed to evaluate the ability of this species to survive at certain low pH peat water levels. Knowing their survival directly or indirectly may be a basis for assessing the adaptation of African catfish in aquaculture, conservation, and restocking in peat waters as part of the peat water ecosystem management.

Estimation of African catfish survival on the mixture of peat and borehole water between 3 and 4 as a result of our treatment is revealed. It showed that the ability to survive African catfish during 96 hours of exposure time to the

mixture of peat and borehole water, the median lethal concentration ( $m$ ),  $LC_{50}$  was about 63.8% in the concentration of peat water. The 95% confidence interval was found between 53.3% and 76.4% in the concentration of peat water (shown in Table 2, calculated from data in Table 1).

In addition to exposure time for both 48 hours and 12 hours, in the same manner, the median lethal concentration ( $LC_{50}$ ) values seemed different from the 96 hours, where the value was more significant with a shorter time (Table 2). As shown in Table 2, the  $LC_{50}$  during 48 hours of exposure time, the value of  $m$  was 70% in the concentration of peat water with the 95% confidence interval, 64.0 - 76.4% peat water. Meanwhile, for the 12-hour exposure time, the  $LC_{50}$  was 78.1 % of peat water with a 95% confidence interval, 69.0-88.4%.

The subject's response during the experiment showed that African catfish fingerlings exposed to peat water above 40% in concentration at the beginning of 1 to 2 hours were often seen rising to the surface like taking oxygen. After 2 hours at a concentration of 70% and above peat water, some of the subjects, catfish, were swimming in a horizontal direction of surface water, and some of them were upright. Then, after 12 hours of exposure to peat water at 70% and 80% in concentration, the fish mortality was 2 inds. (20%) and 5 inds. (50%). Meanwhile, at the same time, for 90% and 100% concentration of peat water, 100% mortality of fish occurred (Table 1). The condition of the dead catfish in 80-100% peat water was run out of mucus, and the skin looked peeling and bruising. In comparison, the survival ability of African catfish (*Clarias gariepinus*) fingerling with a length of 10-15 cm in peat water of low pH might be much better than striped catfish (*Pangasius hypophthalmus*), tilapia (*Oreochromis niloticus*) and carp (*Osphronemus gourami*) fingerling with 3-8 cm in length (Ardianor et al., 2012; Pusparani, 2020). This is likely because of the size of the test fish, in which more large fish seem stronger than smaller ones based on resistance to peat water, and other reasons are possibly due to different species. Mustapha & Mohammed (2018)

reported the resistance of African catfish (*C. gariepinus*) through a simulation they called acid rain using sulfuric acid as a pH regulator of water, making each test medium at pH 3, 4, 5, 6, and 8.01 (control). By applying different sizes of catfish fingerlings (mean 4.1 cm), juveniles (mean 27 cm), and adults (mean 40.2 cm), they got the difference in mortality response. For pH 3 treatment occurred, 100% mortality of catfish (subject) of all life stages. At pH 4, however, especially for juveniles, catfish mortality was found to be 62%, and for fingerling, mortality was about 80%. When we compare the mortality of their juvenile catfish, it was almost close to the result of this study, in which our African catfish mortality was 63.8%. Even though, considering the length (mean 14.2 cm) of catfish, we applied was between the length of fingerlings and juveniles of their catfish.

According to Mustapha & Mohammed (2018), the response of catfish mortality exposed to the low pH of peat water in current research is possible because of reduced oxygen uptake, stress, and circulatory collapse. All of these give rise to behavioural responses and morphological reported the resistance of African catfish (*C. gariepinus*) through a simulation they called acid rain using sulfuric acid as a pH regulator of water, making each test medium at pH 3, 4, 5, 6, and 8.01 (control). By applying different sizes of catfish fingerlings (mean 4.1 cm), juveniles (mean 27 cm), and adults (mean 40.2 cm), they got the difference in mortality response. For pH 3 treatment occurred, 100% mortality of catfish (subject) of all life stages. At pH 4, however, especially for juveniles, catfish mortality was found to be 62%, and for fingerling, mortality was about 80%. When we compare the mortality of their juvenile catfish, it was almost close to the result of this study, in which our African catfish mortality was 63.8%. Even though, considering the length (mean 14.2 cm) of catfish, we applied was between the length of fingerlings and juveniles of their catfish.

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**Table 1.** Mortality of the subject (tests fish), African catfish (*Clarias gariepinus*) (inds.) at various concentrations (density) (%) of peat water (agent).

Exposure time (hour(s))	Concentration of peat water											
	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	
0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	2	5	10	10	
48	0	0	0	0	0	2	3	4	8	10	10	
96	0	0	0	0	1	3	4	6	9	10	10	

**Table 2.** Concentration or density of peat water, the  $LC_{50}$ ,  $m$  (%) exposed to the subject (test fish), African catfish (*C. gariepinus*) at different exposure times with standard error (SE ( $m$ )), confidence level or confidence interval, CI.

Exposure time (hours)	Median Lethal Concentration, $LC_{50}$ ( $m$ ) (%) peat water	SE( $m$ )	95%CI	
			Upper	Lower
12	78.1	1.07	88.4	69.0
48	70.0	1.05	76.4	64.0
96	63.8	1.10	76.4	53.3



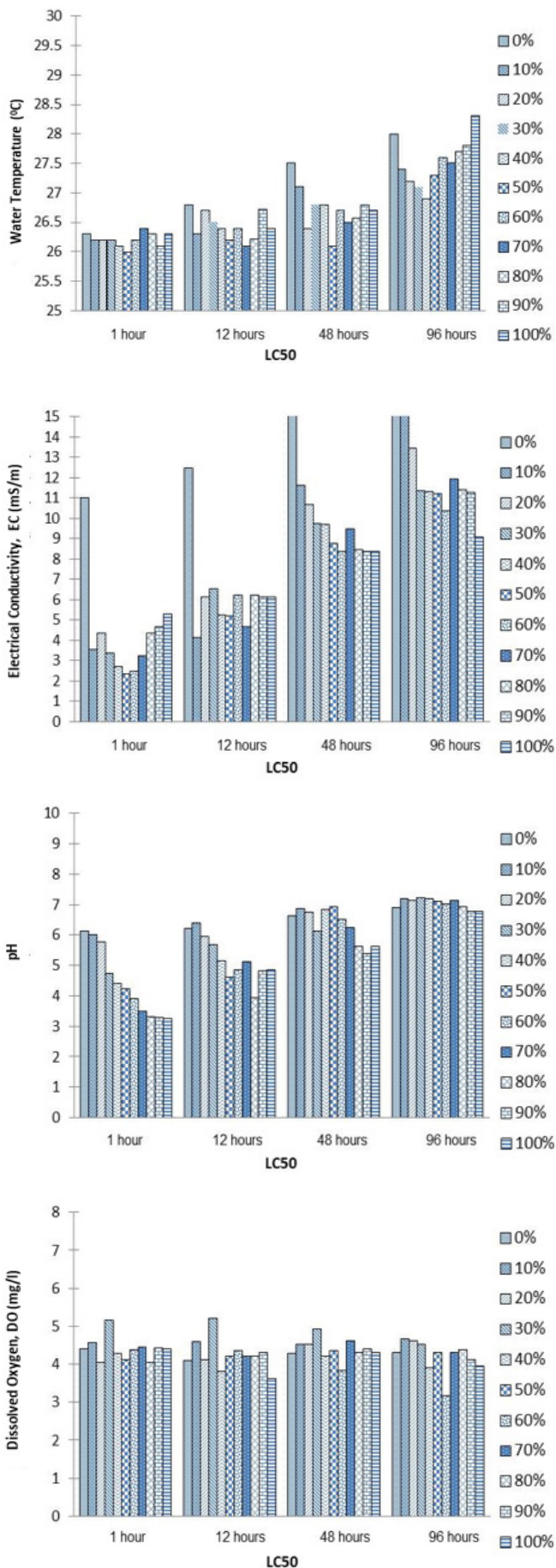


Figure 1. Water quality conditions, i.e. temperature, EC, pH, and DO at different exposure times.

oxygen uptake, stress, and circulatory collapse. All of these give rise to behavioural responses and morphological changes reported the resistance of African catfish (*C. gariepinus*) through a simulation they called acid rain using sulfuric acid as a pH regulator of water, making each test medium at pH 3, 4, 5, 6, and 8.01 (control). By applying different sizes of catfish fingerlings (mean 4.1 cm), juveniles (mean 27 cm), and adults (mean 40.2 cm), they got the difference in mortality response. For pH 3 treatment occurred, 100% mortality of catfish (subject) of all life stages. At pH 4, however, especially for juveniles, catfish mortality was found to be 62%, and for fingerling, mortality was about 80%. When we compare the mortality of their juvenile catfish, it was almost close to the result of this study, in which our African catfish mortality was 63.8%. Even though, considering the length (mean 14.2 cm) of catfish, we applied was between the length of fingerlings and juveniles of their catfish.

According to [Mustapha & Mohammed \(2018\)](#), the response of catfish mortality exposed to the low pH of peat water in current research is possible because of reduced oxygen uptake, stress, and circulatory collapse. All of these give rise to behavioural responses and morphological abnormalities such as erratic swimming, panting and dark bodies, and colouring and lethargy. The morphological responses were almost similar to those we found in this study, where the effect of the low pH of peat water occurred as described previously. Catfish exposed to low pHs, such as peat water seen, tend to suffocate or have difficulty taking oxygen. Although oxygen was relatively available for 96 hours of exposure time, due to the low pH, fish have difficulty breathing through the gills and arborescent, the extra breathing apparatus such as in catfish. According to [Boyd \(1990\)](#), the death of fish at low pH could be because of the hydrogen ion action that affects oxygen uptake, leading to acid stress, proteins precipitation within the epithelial cells, and blood acidosis ([Boyd, 1990](#)). According to [Mustapha & Mohammed \(2018\)](#), fish mortality is possible because of much more mucus production in the gill epithelium, which interferes with the exchange of respiratory gasses and ions across the gill. The acid stress is also morphologically more pronounced on the fish due to the scaleless skin nature, which enables acid to penetrate the skin and be transported along with the body fluid into various internal organs. [Duarte et al. \(2013\)](#) investigated the effect of pH on Na<sup>+</sup> ion uptake in black-water cichlid fish, angelfish (*Pterophyllum scalare*), and discus (*Symphysodon discus*) at low pH of about 3.6. It means that the low pH of freshwater fish may be related to the maintenance ion homeostasis of fish. That mechanism might be similar to the African catfish exposed to low pH of peat water. Unfortunately, we didn't study the ion-regulatory or body salt regulation, suggesting other studies that could focus on such physiological aspects of African catfish on peat water with low pH.

Water quality in [Figure 1](#), the water temperature showed an increase in all experimental units, 0% - 100%, during 96 hours ranging from 26.0°C to 28.3°C, but was still in the optimal range. According to [Prokešová et al. \(2015\)](#), the optimum temperature for African catfish was 22.9 to 30.3°C, similar to [Ogunji & Awoke \(2017\)](#) reported from

26.5 to 31.5°C. For pH, especially from the beginning hour until the 12th hour, as the concentration of peat water increased from 0% to 100%, the pH value respectively decreased. Due to the possibility of the feed pellets effect, pH value tended to increase during 96 hours of exposure time, as shown in Figure 2. Thus, the increment of pH was about 2.6 digits for the average of experimental units. The artificial fish feed can affect the increased pH of water, according to Dewanggani et al. (2021), because it contains ash of about 12% as an Indonesian feed factory (<https://mataharisakti.com/products/prima-feed-lp>), and in line with Gracia Lovez et al. (2002) that ash content of artificial fish feed reach 16% with calcium about 3.5%. EC's electrical conductivity fluctuated, ranging from 2.38 to 20.90 mS/cm. Its value tended to increase during exposure, with an average of 8.2 digits. Unlike temperature, pH, and EC, DO seemed not to change from the 1st hour to the 96th hour for all experimental units. Each experimental unit was installed with an aerator that regularly supplies oxygen (Figure 2). The DO concentration ranged from 3.18 to 5.22 mg/l, which was still in the optimal range for African catfish according to Oyo & Ekanem (2011), Primaningtyas et al. (2015), and Žibiene & Žibas (2017).

The fish mortality and the changes in pH values due to increasing peat water concentration at each primary observation time, i.e. 1, 12, 48, and 96 hours are shown in Figure 2. The increase of peat water concentration from 0% to 100% has caused a decrease in pH and catfish mortality, mainly before 12 hours of exposure time. The decrease of pH, in the beginning, seemed to have a substantial effect on the mortality of catfish for the next exposure time, such as 48 and 96 hours, even though pH value tended to increase slowly afterwards. This also is clearly shown in Figure 3.

Figure 3 shows the change in pH value and mortality of fish at each concentration, 0-100%, as the increase of exposure time. It was seen that from 1 to 8 hours of exposure time, in particular for the concentration of peat water from 60 to 100%, the pH value slightly increased with a narrow range, from 3.25 to 4.18. The effect of low pH on fish at this beginning of time caused their stress and mortality for the next exposure time, although pH gradually increased. The low pH of the mixed Peat and borehole water generally is caused by the humic compound such as humic and fulvic acid contained in the peat water (Steinberg, 2003; Ishikawa et al., 2005).

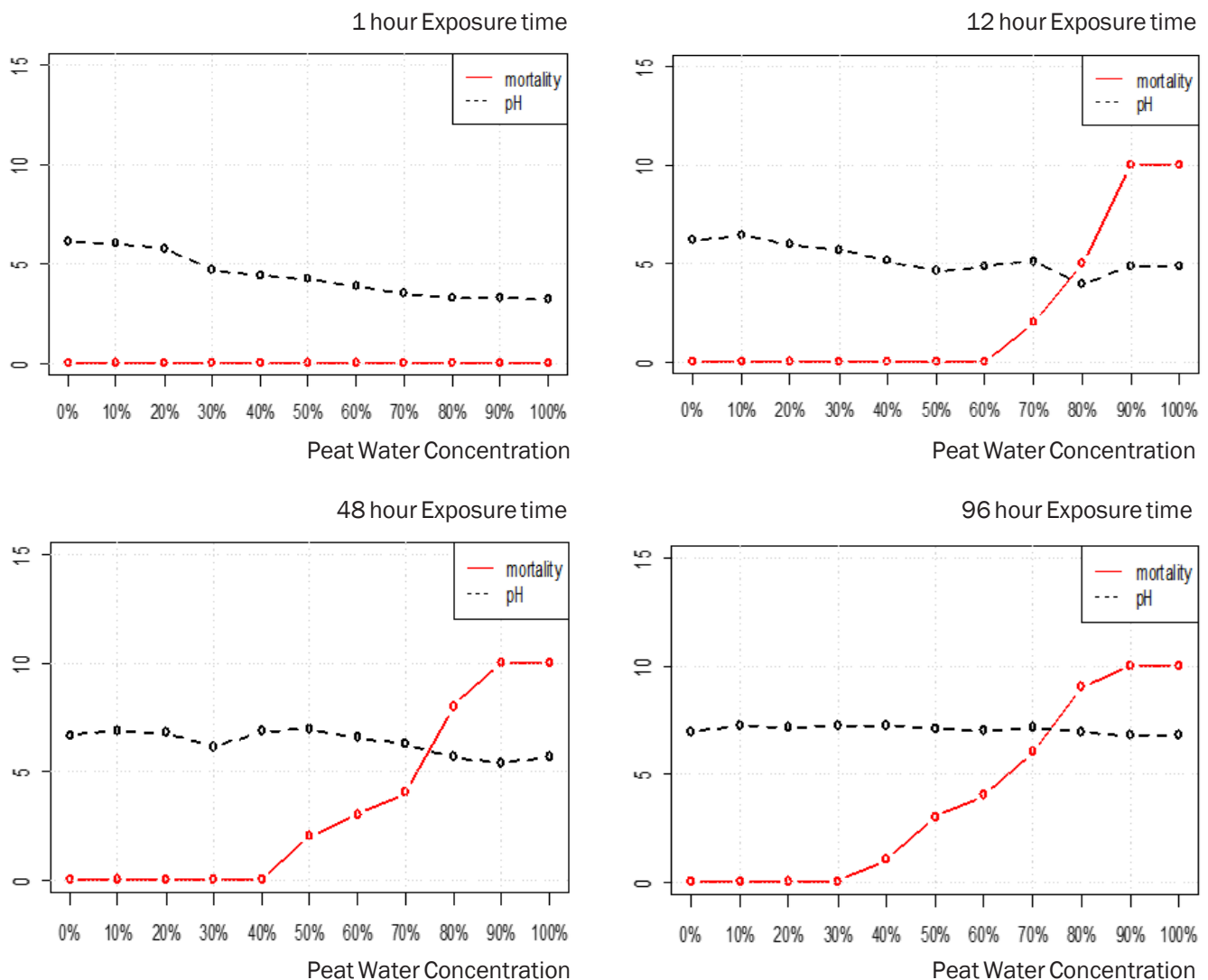


Figure 2. The fish mortality (inds.) and the changes in pH values as the increase of peat concentration at the exposure times of 1, 12, 48, and 96 hours.

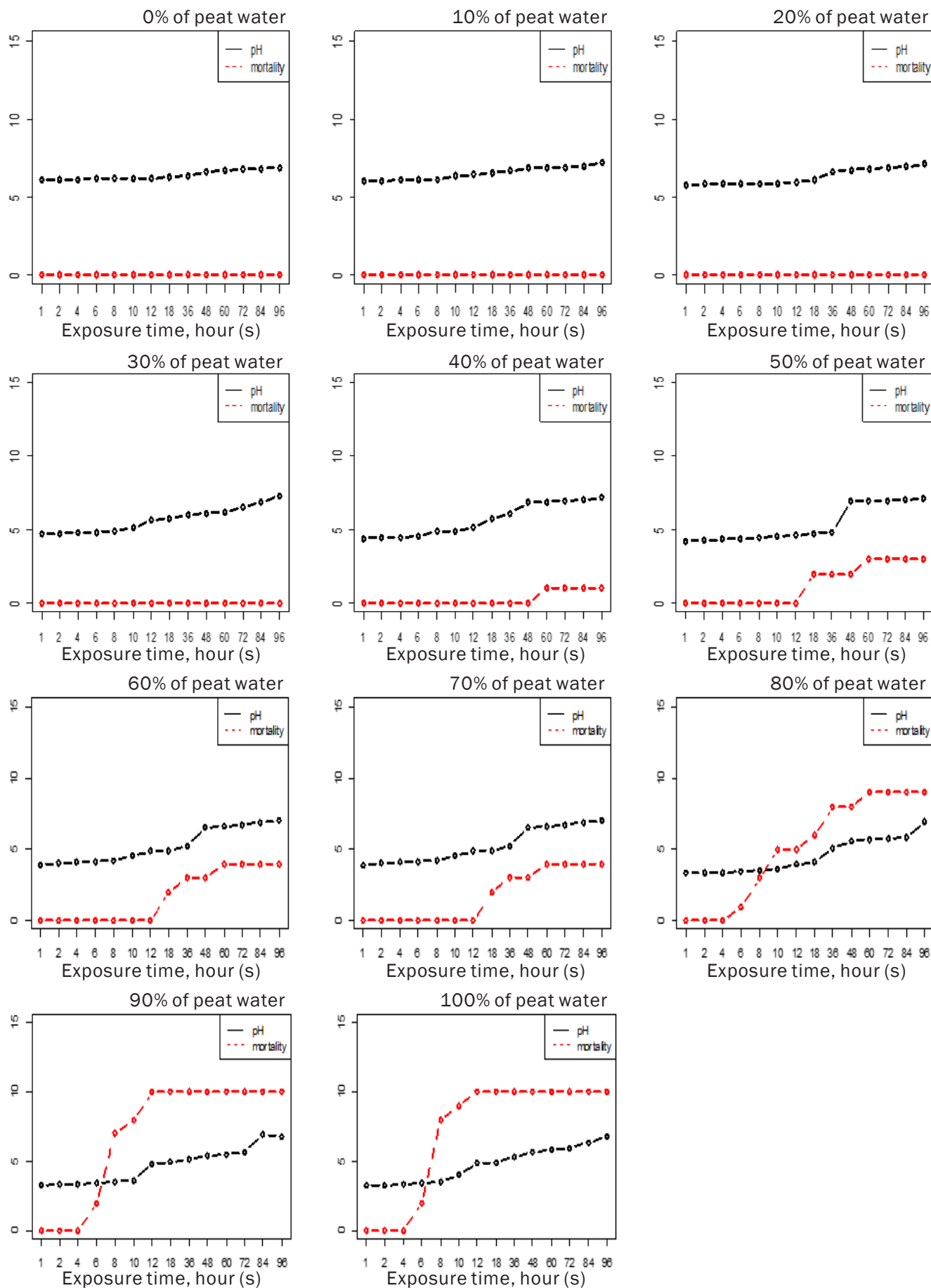


Figure 3. The changes of pH and catfish mortality (inds.) for each concentration along the exposure time. The fish mortality line of each time is an aggregate number.



## CONCLUSIONS

The maximum survival of African catfish in the low pH of peat water was about 63.8% in concentration by LC<sub>50</sub> 96 hours. The lethal response of the African catfish to such peat water before death was rising to the surface, swimming horizontally, and then vertically floating near the water surface. After death, the fish was run out of mucus, and the skin looked peeling and bruising.

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