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AQUICULTURE

Effect of simulated acid rain on the survival, mortality, behaviour and morphology of African mud catfish *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT. This study simulated acidic habitat for different life stages of *Clarias gariepinus* to know the effect on survival, mortality, morphology and behaviour. The fish were exposed to pHs 3, 4, 5, 6 and 8.01 (control) for 35 days. 100% mortality was recorded for all the life stages in pH 3, and 80, 62 and 36% of mortality of fingerlings, juveniles and adults respectively were recorded in pH 4. In pH 5, 30, 26 and 12% mortalities of fingerlings, juveniles and adults respectively were recorded and at pH 6 mortalities of fingerlings, juveniles and adults were 12, 10 and 4% respectively. Control pH mortalities were 10, 6 and 2% for fingerlings, juveniles and adults respectively. Mortality was due to lack of oxygen uptake, stress and circulatory collapse. All these elicited the behavioural responses and morphological abnormalities like erratic swimming, gasping, dark body colouration and lethargy. The study showed that different life stages of *C. gariepinus* tolerate different acidic pH waters, with high mortalities of the fingerlings, juveniles and adults in pHs 3, pH 4 is sub-lethal to fingerlings, juveniles and adult, pH 5 sub-lethal to fingerlings and sub-optimal for juveniles, and pH 6 optimal for all life stages.

Keywords: fingerlings, juveniles, adults, pH, stress, sulphur oxides.

Efeito da chuva ácida simulada sobre a sobrevivência, mortalidade, comportamento e morfologia do peixe-gato de lama africana *Clarias gariepinus* (Burchell, 1822)

RESUMO. Este estudo simulou um habitat ácido para diferentes estágios de vida de *Clarias gariepinus* para conhecer o efeito na sobrevivência, mortalidade, morfologia e comportamento. Os peixes foram expostos aos pH 3, 4, 5, 6 e 8.01 (controle) por 35 dias. A mortalidade de 100% foi registrada em todos os estágios da vida em pH 3, e 80, 62 e 36% da mortalidade de alevinos, juvenis e adultos, respectivamente, foram registradas em pH 4. Em pH 5, 30, 26 e 12% de mortalidade de alevinos, juvenis e adultos, respectivamente, foram registrados e, a pH 6, a mortalidades de aletanas, juvenis e adultos foram 12, 10 e 4%, respectivamente. A mortalidade por pH de controle foi de 10, 6 e 2% para alevinos, juvenis e adultos, respectivamente. A mortalidade deveu-se à falta de absorção de oxigênio, estresse e colapso circulatório. Tudo isso provocou respostas comportamentais e anormalidades morfológicas como natação errática, ofegante, coloração do corpo escuro e letargia. O estudo evidenciou que diferentes estágios de vida de *C. gariepinus* toleram diferentes águas ácidas do pH, com alta mortalidade de alevinos, juvenis e adultos em pH 3, pH 4 é sub-letal para alevinos, juvenis e adultos, pH 5 sub-letal para alevinos e sub-ótima para juvenis, e pH 6 ideal para todos os estádios da vida.

Palavras-chave: alevinos, juvenis, adultos, pH, estresse, óxidos de enxofre.

Introduction

Acid rain is a term used to describe the coming in contact of rain water with oxides of sulphur and nitrogen which significantly makes the rain water highly acidic. Natural unpolluted rainwater is slightly acidic as a result of carbon dioxide dissolving in it. Rainwater becomes more acidic with the pollution of sulphur dioxide and nitrogen oxides to form sulphuric and nitric acids respectively. These pollutants come from burning of fossil fuels in thermal power stations, burning of gasoline in vehicles and heavy equipment and from smelting operations in oil industries and manufacturing industries (Singh & Agrawal, 2008).

The effect of acid rain, acid deposition and increasing acidity of lakes on fish has been documented by various authors (Jensen & Snekvik, 1972, Fromm, 1980, Alabaster & Llyod, 1980, Ikuta, Amano, & Kitamura, 1999). With urbanization and increasing population especially in developing countries and with no effective act or legislation to reduce these pollutants emission, the problems of acidification of rivers, streams, lakes, ponds and reservoirs which hitherto has not been fully quantified, will manifest in the near future and continue to magnify resulting in declining population of fish species in these aquatic ecosystems.

The objectives of this work is therefore to simulate acidic water and acidification of the habitat of African mud catfish *Clarias gariepinus* (a very important food and economical fish in Africa) in order to look at the effect and time duration of different acidic pHs on the survival, mortality, morphology and behaviour of the different life stages (fingerlings, juveniles and adult) of the species in the subsequent event of acid rain and acidification of their habitat.

Material and methods

Four experimental pHs (3, 4, 5 and 6) and a controlled pH (8.01) were used for the experiment. Only 98% concentrated sulphuric acid was used to simulate acidic water and acidification of the culture media rather than the combination of sulphuric and nitric acids. This is to reduce the number of variables being introduced in the experiment as well as H_2SO_4 being the largest contributor to acid rain (Singh & Agrawal, 2008). The sulphuric acid was added to the culture water to make stock pH-adjusted solution water. This was to ensure that the pH would not change over the period of the experiment due to evaporation, precipitation or with the addition of the water. 40 L each of pH 3, 4, 5 and 6 of the stock solution were prepared by serial dilution with the acid and the culture water using a Hanna portable pH/EC/TDS/Temperature combined waterproof tester/meter model HI 98129. The control pH was a borehole water having a pH of 8.01.

A total of 450 *C. gariepinus* was used for the experiment comprising of fingerlings (average weight and length of 3.6 g and 4.1 cm), juveniles (average weight and length of 100 g and 27 cm) and adults (average weight and length of 500 g and 40.2 cm). 10 each of the fingerlings, juveniles and adults were stocked in 40 L tank (1 x 1 x 0.2 m) of acidified water of pH 3, 4, 5, and 6 as well as the control with a base pH of 8.01. Each of the tanks was replicated in triplicates. They were first acclimatized under laboratory condition for 7 days before the start of the experiment. The fishes were fed with coppens fish feed twice daily (8.00 am and 6.00 pm) at 3% of their biomass. The experiment was conducted for 35 days. Survival and mortality of the fish were evaluated daily with the counting of surviving and dead fish in each tank. Tactile and visual observations were carried daily out on the fish to observe morphological and behavioural changes that occurred due to the effect of the different concentration of the acids on the different life stages of the fish as described by Mustapha (2017).

Data were analyzed using chi-squared test and Wald test of regression coefficients with significant difference observed at $p < 0.05$. SPSS 20.0® statistical package (Armonk, New York, USA) was used for the data analysis.

Result

100% mortality of all the life stages of *C. gariepinus* was recorded in pH 3 tanks (Table 1).

In pH 4 tanks, 40, 62 and 36% mortalities of the fingerlings, juveniles and adults were recorded (Table 1). In the pH 5 tanks, 30% mortality of the fingerlings were observed, while 26% mortality of the juveniles and 12% mortality of the adults were recorded (Table 1). In pH 6 tanks, 12% mortality of the fingerlings were recorded, with 10% and 4% mortalities of the juveniles and adults were observed (Table 1).

10% mortality of the fingerlings, 6% mortality of the juveniles and 4% mortality of the adults were recorded in the control pH 8.01 (Table 1). There was significant difference ($p < 0.05$) in the survival and mortality among the acidic pHs and the control pH. The regression coefficient shows that adults will survive more than the juveniles and juveniles will survive more than the fingerlings in the different acidic pHs. The survival trend is Adult > Juvenile > Fingerling and the mortality is in the reverse order of Adult < Juvenile < Fingerling.

Table 1. Survival and mortality of fingerling, juvenile and adult of *C. gariepinus* in different acidic pH and control pH.

pH	Fingerling survival %	Fingerling mortality %	Juvenile survival %	Juvenile mortality %	Adult survival %	Adult mortality %
3	0^a	100^a	0^a	100^a	0^a	100^a
4	20^b	80^b	38^b	62^b	64^b	36^b
5	70^c	30^c	74^c	26^c	88^c	12^c
6	88^d	12^d	90^d	10^d	96^c	4^d
Control 8.01	90^d	10^d	94^d	6^d	98^c	2^d

Values with different superscript are significantly different ($p < 0.05$).

Morphological and behavioural changes observed in the fish include erratic swimming among juveniles and adults in especially in pHs 4 and 5, accelerated operculum movement leading to gasping, barbells vibration and frequent coming to the surface. Very dark body colouration and slimy mucous secretions were observed on the gills and body surface; peeling and skin erosion were also noted. There was bleeding from the dorsal fins and bending of the caudal fin, some fins became disintegrated and impairment in feeding was observed. In pH 4 and 5 tanks, fingerlings were lethargic, confined to the bottom of the tanks and only moved when agitated with the movement being in one direction. Feeding was observed to be reduced with uneaten food in the tanks (Tables 2, 3 and 4).

Table 2. Morphological and behavioural observations of fingerling of *C. gariepinus* in different acidic pH and control.

Observations	pH 3	pH 4	pH 5	pH 6	Control
Morphological	None as mortality was recorded	Very dark body colouration, slimy mucous secretions on the gills and body surface; peeling and skin erosion, bleeding from the dorsal fins and bending of the caudal fin, some fins became disintegrated	Very dark body colouration, slimy mucous secretions on the gills and body surface; peeling and skin erosion, bleeding from the dorsal fins and bending of the caudal fin, some fins became disintegrated	Normal body colouration except some few dark patches, no morphological abnormalities observed.	Normal body colouration, no morphological abnormalities seen
Behavioural	None as mortality was recorded	Lethargy, they were confined to the bottom of the tanks and only moved when agitated with the movement being in one direction, feeding impaired	Lethargy, they were confined to the bottom of the tanks and only moved when agitated with the movement being in one direction, feeding impaired	Movement not impaired, they were seen on the surface of tanks, no abnormal behavioural activities observed	Normal movement and behavioural responses observed

Table 3. Morphological and behavioural observations of juvenile of *C. gariepinus* in different acidic pH and control.

Observations	pH 3	pH 4	pH 5	pH6	Control
Morphological	None as mortality was recorded	Very dark body colouration, slimy mucous secretions on the gills and body surface; peeling and skin erosion, bleeding from the dorsal fins and bending of the caudal fin, some fins became disintegrated	Very dark body colouration, slimy mucous secretions on the gills and body surface; peeling and skin erosion, bleeding from the dorsal fins and bending of the caudal fin, some fins became disintegrated	Normal body colouration except some few dark patches, no morphological abnormalities recorded.	Normal body colouration, no morphological abnormalities seen
Behavioural	None as mortality was recorded	Erratic swimming, accelerated operculum, gasping, barbells vibration and frequent coming to the surface, feeding impaired	Erratic swimming, accelerated operculum, gasping, barbells vibration and frequent coming to the surface, feeding impaired	No behvioural abnormalities observed.	Normal movement and behavioural responses observed

Table 4. Morphological and behavioural observations of adult of *C. gariepinus* in different acidic pH and control.

Observations	pH 3	pH 4	pH 5	pH 6	Control
Morphological	None as mortality was recorded	Very dark body colouration, slimy mucous secretions on the gills and body surface; peeling and skin erosion, bleeding from the dorsal fins and bending of the caudal fin, some fins became disintegrated	Very dark body colouration, slimy mucous secretions on the gills and body surface; peeling and skin erosion, bleeding from the dorsal fins and bending of the caudal fin, some fins became disintegrated	Normal body colouration, no morphological abnormalities recorded.	Normal body colouration, no morphological abnormalities seen
Behavioural	None as mortality was recorded	Erratic swimming, accelerated operculum, gasping, barbells vibration and frequent coming to the surface, feeding impaired	Erratic swimming, accelerated operculum, gasping, barbells vibration and frequent coming to the surface, feeding impaired	No behvioural abnormalities observed.	Normal movement and behavioural responses observed

Discussion

Acid rain or acid deposition is a serious problem affecting fish and fisheries in many parts of the western world. In no time, the effects on fish and fisheries would also be felt in developing world due to increasing population, urbanization and modernization. Several factors such as species (Palmer, Klauda, & Lewis, 1988), stock and strain (Robinson, Dunson, Wright, & Mamolito, 1976) and life stage (Kwain & Rose, 1985) are known to influence acidic toxicity based on acid deposition on fishes.

Despite the acclimatization of the different life stages of *C. gariepinus* under laboratory conditions, the effects of the acidic media was strongly felt on the fish in terms of survival, mortality, morphology and behaviour. The death of fingerlings, juveniles and adults in pH 3 could be due to toxic action of hydrogen ions which affected oxygen uptake leading to acid stress, precipitation of proteins within the epithelial cells; and acidosis of the blood (Boyd, 1990). It could also be probably attributable to erosion of the epidermal layer of the integument and gills, brain, kidney and spleen injury, lysis of erythrocytes as reported by (Daye & Garside, 1980; Hill, Foley, Blazer, Werner, & Giannon, 1988), and failure of the ionoregulatory system leading to circulatory collapse (Peterson & Martin-Robichaud, 1986). Incomplete development of the ion regulation system among the fingerlings might have underlain their reduced acid tolerance (Daye & Garside, 1980). Ndubuisi, Chimezie, Chinedu, Chikwem and Alexander (2015) reported 100% mortality of fingerlings of *Clarais gariepinus* in pH 3.

The pH tolerance of fish has been shown to increase with age of the fish (Rask, 1984). The largest survival of juveniles and adults of *C. gariepinus* in pHs 4, 5 and 6 was attributed to their efficient ion transportation systems which allowed them to maintain a more efficient salt balance than the fingerlings, while mortality could be as a result of ionoregulatory failure (Peterson & Martin-Roblchaud, 1986) in body salt regulation leading to

haemoconcentration and circulatory collapse (Mount, Hockett, & Gern, 1988). The mortality could also be due to the production of mucus on the gill epithelium, which interferes with the exchange of respiratory gasses and ions across the gill.

All the effects of the acid stress mentioned above on the fish species elicited the behavioural responses and morphological abnormalities seen among the different life stages. The morphological effects were more pronounced on the fish due to the scaleless nature of the skin which allowed the acid to penetrate into the skin and be transported along the body fluid into various organs of the body. The survival and mortality recorded in the species could be linked to the morphological and behavioural responses of the fish in the different acidic media.

The United States Environmental Protection Agency [USEPA] (1986) concluded that a pH range of 6.5 to 9.0 provides adequate protection for the life of freshwater fish. Outside this range, fish suffer adverse physiological effects that increase in severity as the degree of deviation increases until lethal levels are reached. Alabaster and Lloyd (1980) identified the pH range that is not directly lethal to freshwater fish as 5.0-9.0.

Conclusion

This study has shown that different life stages of *C. gariepinus* tolerate different acidic pH waters with high mortalities of the fingerlings and juveniles in pHs 3 and 4, while adults could survive in pH 4 and not in pH 3. Generally, all the life stages could thrive in pHs 5 and 6, with pH 3 lethal to all life stages, pH 4 sub-lethal to fingerlings, juveniles and adult, pH 5 sub-lethal to fingerlings and sub-optimal for juveniles, and pH 6 optimal for all life stages. This low acid tolerance of *C. gariepinus* tends to confirm the fish as a hardy species in spite of its scaleless body compared to other tropical freshwater fish species.

References

Alabaster, J. S., & Lloyd, R. (1980). *Water quality criteria for freshwater fish*. London, UK: Butterworths.

Boyd, C.E. (1990). *Water quality in ponds for aquaculture*. Auburn, AL: Birmingham Publishing.

Daye, P. G., & Garside, E. T. (1980). Structural alterations in embryos and alevins of the Atlantic salmon, *Salmo salar* L. induced by continuous or short term exposure to acidlc levels of pH. *Canadian Journal of Zoology, 58*(1), 27-43.

Fromm, P. O. (1980). A review of some physiological and toxicological responses of freshwater fish to acid stress. *Environmental Biology of Fishes, 5*(1), 79-93.

Hill, J., Foley, R. E., Blazer, V. S., Werner, R. G., & Giannon, J. E. (1988). Effects of acidic water on young-of-the-year smallmouth bass (*Micropterus dolomieui*). *Environmental Biology of Fish, 21*(3), 23-29.

Ikuta, K., Amano, M., & Kitamura, S. (1999). Effects of acid rain on inland water ecosystem - effects on fish. *Kankyokagakukaishi, 12*(2), 259-264.

Jensen, K. W., & Snekvik, E. (1972). Low pH levels wipe out salmon and trout populations in southernmost Norway, *Ambio, 1*(6), 223-225.

Kwain, W., & Rose, G. A. (1985). Growth of brook trout, *Salvenlinus fontinalis*, subject to sudden reduction of pH during their early life history. *Transactions of the American Fisheries Society, 114*(1), 564-570.

Mount, D. I., Hockett, J. R., & Gern, W. A. (1988). Effects of long-term exposure to acid, aluminum, and low caicium on adult brook trout (*Salvenlinus fontinalis*). 2. Vitellogensis and osmoregulation. *Canadian Journal of Fisheries and Aquatic Sciences, 45*(9), 1633-1642.

Mustapha, M. K. (2017). Dynamics of hexavalent chromium in four types of aquaculture ponds and its effect on the morphology and behaviour of cultured *Clarias gariepinus* (Burchell, 1822). *Toxicological Research, 33*(2), 119-124.

Ndubuisi, C. U., Chimezie, J. A., Chinedu, C. U., Chikwem, C. I., & Alexander, U. (2015). Effect of pH on the growth performance and survival rate of *Clarias gariepinus* fry. *International Journal of Research in Biosciences, 4*(3), 14-20

Palmer, R. E., Klauda, R. J., & Lewis, T. E. (1988). Comparative sensitivities of bluegill, channel catfish, and fathead minnow to pH and aluminum. *Environmental Toxicology and Chemistry, 7*(6), 505-516.

Peterson, R. H., & Martin-Robichaud, D. S. (1986). Growth and major inorganic cation budgets of Atlantic salmon alevins at three ambient acidities. *Transactions of the American Fisheries Society, 115*(2), 220-226.

Rask, M. (1984). The effect of low pH on perch, *Perca fluviatilis* L. II. The effect of acid stress on different development stages of perch. *Annales Zoologici Fennici, 21*(1), 9-13.

Robinson, G., Dunson, W., Wright, J., & Mamolito, G. (1976). Difference in low pH tolerance among strains of brook trout (*Salvenlinus fontinalis*). *Journal of Fish Biology, 8*(1), 5-17.

Singh, A., & Agrawal, M. (2008). Acid rain and its ecological consequences. *Journal of Environmental Biology, 29*(1), 15-24.

United States Environmental Protection Agency [USEPA]. (1986). *Quality criteria for water 1986*. Washington, D.C.: Environmental Protection Agency; Office of Water Regulations and Standards.

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