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

Effect of acidity on *Clarias gariepinus*

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Abstract

Increasing industrialization in developing countries will bring acid rain which is the contamination of rain water with oxides of sulphur and nitrogen. The effect could be devastating on fish. 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. The pHs were prepared by serial dilution with sulphuric acid and deionized water. 100% mortality was recorded for all the life stages in pH 3, 80%, 62% and 36% mortalities 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 noted and 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.

Key words: fingerlings, juveniles, adults, pH, stress, sulphur oxides

Abstrato

O aumento da industrialização nos países em desenvolvimento trará chuva ácida, que é a contaminação da água da chuva com óxidos de enxofre e nitrogênio. O efeito pode ser devastador para os peixes. Este estudo simulou habitat ácido para diferentes estádios de vida de Clarias gariepinus para conhecer o efeito sobre a sobrevivência, mortalidade, morfologia e comportamento. Os peixes foram expostos a pH 3, 4, 5, 6 e 8,01 (controlo) durante 35 dias. Os pH foram preparados por diluição em série com ácido sulfúrico e água desionizada. A mortalidade de 100% foi registrada para todos os estádios de vida em pH 3, 80%, 62% e 36% de mortalidade de alevinos, juvenis e adultos, respectivamente, foram registrados em pH 4. Em pH 5, 30%, 26% e 12% Alevinos, juvenis e adultos, respectivamente, ea mortalidade de 6, 10 e 4%, respectivamente, de juvenis e adultos. As mortalidades de pH de controlo foram de 10%, 6% e 2% para alevinos, juvenis e adultos respectivamente. A mortalidade foi devido 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 mostrou que os diferentes estádios de vida de C. gariepinus toleram diferentes águas de pH ácido.

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 rain water is slightly acidic as a result of carbon dioxide dissolving in it. Rain water 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.

The effect of acid rain, acid deposition and increasing acidity of lakes on fish has been documented by various authors (Jensen and Snekvik 1972, Fromm 1980, Alabaster and Llyod, 1980, Ikuta *et al*. 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 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.

Materials and Methods

Four experimental pHs (3, 4, 5 and 6) and a control pH (8.01) were used for the experiment. Only 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 H2SO4 being the largest contributor to acid rain (Singh and Agrawal, 2008). The sulphuric acid was added to deionized 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 deionized water. 40 litres each of pH 3, 4, 5 and 6 of the stock solution were prepared by serial dilution with the acid and deionized water using a Hanna portable pH/EC/TDS/Temperature combined water proof tester/meter model HI 98129. The control pH was a borehole water having a pH of 8.01.

A total of 450 *Clarias gariepinus* was used for the experiment comprising of fingerlings (average weight and length of 3.6g and 4.1cm), juveniles (average weight and length of 100g and 27.cm) and adults (average weight and length of 500g and 40.2cm). 10 each of the fingerlings, juveniles and adults were stocked in 40 litres tank (1x1x0.2m) 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 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.

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

No survival was recorded among the fingerlings of *C. gariepinus* stocked in the pH 3 tanks as all of the 50 died on the day 1. 100% mortality and 0% survival of the fingerlings was recorded in pH 3 tanks within the 35 days of the experiment. A total of 40 juveniles died on day 2, while the remaining 10 died on day 4 in pH 3 tanks. 100% mortality and 0% survival of the juveniles was recorded in pH 3 tanks within the 35 days of the experiment. 30 adults were found dead on day 5 and the remaining 20 died on day 6 in the pH 3 tanks. 100% mortality and 0% survival of the adults was recorded in pH 3 tanks within the 35 days of the experiment. Thus, There were no life stages of the fish that survived the 35 days of the experiment in pH 3 (Table 1).

In pH 4 tanks, 10 fingerlings mortalities were recorded on day 8, while another 22 deaths were observed on day 17. On day 32, 5 deaths occurred while 3 mortalities were recorded on day 34. 10 fingerlings survived in pH 4 tanks till the 35 days of the experiment. 20% survival and 40 % mortality of the fingerlings were recorded in pH 4 tanks during the experimental days. As for the juveniles in pH 4 tanks, 5 deaths were recorded on day 8, 16 mortalities occurred on day 19, while 10 deaths were observed on day 33. 19 juveniles survived the 35 experimental days in pH 4, translating to 38% survival and 62 % mortality of the juveniles recorded in pH 4 tanks during the experimental days. 6 adult deaths were noted on day 10, while 12 adult mortalities were observed on day 34. 32 adults were seen to have survived the 35 days of the experiment in pH 4 tanks, thus, 64% survival and 36 % mortality of the adults were recorded in pH 4 tanks during the experimental days (Table 1).

In the pH 5 tanks, 6 fingerlings mortalities were recorded on day 8, while 5 and 4 mortalities were observed on days 33 and 34 respectively. 35 fingerlings were noted to have survived in pH 5 till the end of the experiment making it 70% survival and 30 % mortality of the fingerlings recorded in pH 5 tanks during the experimental days. 5 juvenile deaths were noted on day 10, while 8 more died on day 33. A total of 37 juveniles survived till the end of the experiment in pH 5, this translated to 74% survival and 26 % mortality of the juveniles observed in pH 5 tanks during the experimental days. 6 adult deaths were recorded on day 17 and 44 adults survived till the end of the experiment in pH 5 tanks, which was 88% survival and 12 % mortality of the adults recorded in pH 5 tanks during the experimental days (Table 1).

In pH 6 tanks, fingerling death recorded was 2 on day 8, and 4 more deaths of the fingerlings were observed on day 34. The total number of fingerlings that survived the experiment in pH 6 was 44. 88% survival and 12 % mortality of the fingerlings were recorded in pH 6 tanks during the experimental days. Juvenile mortalities were 3 and 2 respectively recorded on days 17 and 34. Thus number of juveniles’ survival at pH 6 was 45 making it 90% survival and 10 % mortality of the juveniles recorded in pH 6 tanks during the experimental days. For the adults, 2 deaths were recorded on day 18, while 48 survived the experiment in the pH 6 tanks, thus 96% survival and 4% mortality of the adults were observed in pH 6 tanks during the experimental days (Table 1).

A total mortality of 5 fingerlings died on days 5 and 7; 3 death of the juveniles on days 17 and 25 were noted and 1 adult death occurred on day 8 from the control pH of 8.01. 45 fingerlings, 47 juveniles and 49 adults survived till the end of the experiment in the control pH 8.01. Thus, 90% survival and 10% mortality of the fingerlings, 94% survival and 6% mortality of the juveniles and 98% survival and 2% mortality of the adults were recorded in the control pH 8.01 tanks during the experimental days (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  survival % | Fingerling  mortality | Fingerling  mortality % | Juvenile  survival | Juvenile  survival % | Juvenile  mortality | Juvenile  mortality % | Adult survival | Adult survival % | Adult mortality | Adult mortality % |
| 3 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 100 |
| 4 | 10 | 20 | 40 | 80 | 19 | 38 | 31 | 62 | 32 | 64 | 18 | 36 |
| 5 | 35 | 70 | 15 | 30 | 37 | 74 | 13 | 26 | 44 | 88 | 6 | 12 |
| 6 | 44 | 88 | 6 | 12 | 45 | 90 | 5 | 10 | 48 | 96 | 2 | 4 |
| Control 8.01 | 45 | 90 | 5 | 10 | 47 | 94 | 3 | 6 | 49 | 98 | 1 | 2 |

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 pH

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observations | Fingerling  Ph 3 | Fingerling  Ph 4 | Fingerling  Ph 5 | Fingerling  Ph 6 | fingerling  ph 8.01 (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 pH

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observations | Juvenile  Ph 3 | Juvenile  Ph 4 | Juvenile  Ph 5 | Juvenile  Ph 6 | Juvenile  ph 8.01 (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 pH

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observations | Adult  Ph 3 | Adult  Ph 4 | Adult  Ph 5 | Adult  Ph 6 | Adult  ph 8.01 (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 are known to influence acidic toxicity based on acid deposition on fishes which include species (Palmer *et al*. 1988), stock and strain (Robinson *et al*. 1976) and life stage (Kwain and Rose 1985).

In spite of the acclimatization of the different life stages of *Clarias 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/or acidosis of the blood (AFS 1982; 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 and Garside 1980; Hill *et al*. 1988), and failure of the ionoregulatory system leading to circulatory collapse (Peterson and Martin-Robichaud 1986). Incomplete development of the ion regulation system among the fingerlings might have underlie their reduced acid tolerance (Daye and Garside 1980). Ndubusi *et al*. (2015) reported 100% mortality of fingerlings of *Clarais gariepinus* in pH 3.

pH tolerance of fish has been shown to increase with age of the fish (Rask, 1984). The increased 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 effective salt balance than the fingerlings, while mortality could be as a result of ionoregulatory failure (Peterson and Martin-Roblchaud 1986) in body salt regulation leading to haemoconcentration and circulatory collapse (Mount *et al*. 1988). The mortality could also be due to production of mucus on the gill epithelium, which interferes with the exchange of respiratory gasses and ions across the gill. Hence, respiratory distress and osmotic imbalance were culpable as the primary physiological symptoms that lead to acid stress in the fish.

All the effects of the acid stress mentioned above on the fish species elicited the behavioural responses and morphological abnormalities such as erratic swimming, accelerated operculum movement leading to gasping, barbells vibration, very dark body colouration and slimy mucous secretions on the gills and body surface, peeling and erosion of the skin, bleeding from the dorsal fins, bending of the caudal fin, and disintegration of some fins, impairment in feeding and lethargy 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. Ellgaard and Gillmore (1975) reported that bluegill larvae were less active at pH levels of 5.5 and the swimming was erratic, while Jordhal and Benson (1987) noted that domestic brook trout exhibited lethargic and/or uncoordinated swimming behaviour in response to acid exposure. Jones *et al*. (1987) observed decreased attraction to food, reduced feeding, hyperactivity as some of the behavioural responses of fish to acid conditions. 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 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.

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.

Conclusion

Although acid deposition and acid rain has not been well documented in many developing countries, there is no doubt that the phenomenon is present and on-going in the aquatic ecosystems of these countries. Researches should be geared towards understanding the phenomenon and its effects on water bodies and their biota.

A mitigating measure to the problem in the event of its occurrence is the reduction of emissions of oxides of sulphur and nitrogen. This could only be achieved through enactment of laws and implementation of acid pollution control strategies as done in developed countries. However, in the case, the lakes, rivers, streams, ponds and reservoirs have been identified as been acidic, liming of these water bodies could be done to raise the pH in order to remediate the fish populations from the effects of the acidity.

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