Nigerian Journal of Pure and Applied Sciences
NJPAS

Influence of Different levels of Water pH on Survival rates of Laboratory reared *Clarias anguillaris* Fingerlings in Minna, Nigeria.

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Abstract
A 42 days study was conducted to determine the influence of different levels of water pH on survival rates of *Clarias anguillaris* fingerlings. The experiment consisted of 4 treatments with 2 replicates each in a complete randomized design and stocked with fifty 28 days old *Clarias anguillaris* fingerlings. Eight plastic indoor aquaria tanks (53 x 35 x 22) cm^3 were filled with *dechlorinated* borehole water at pH 4, 5, 6, 7.3 (control). The stock solutions of 2% concentrated Hydrochloric acid (HCL) and 0.01 M of sodium Hydroxide solution were used to determine the pH treatments. Physicochemical parameters were determined weekly, while the survival rates were recorded daily. Fingerlings were fed twice daily to satiation, while the exchange of water and fixing of the treatments were done twice a week. Results showed that there were no significant of differences ($P > 0.05$) in the survival rates of *C. anguillaris* fingerlings cultured in pH 6.0 and pH 7.30. Weekly survival rates and the dissolved oxygen concentration were significantly lowest ($P<0.005$) at pH 4.0. Biochemical oxygen demand concentration (2.50 ± 0.17mg/L) at pH 4.00 was significantly higher ($P< 0.005$). The optimal pH range for *C. anguillaris* fingerlings was within 6.0 to 7.3 (control).

Keywords: Water pH, *Clarias anguillaris*, survival rates, and physicochemical parameters

Introduction

It has been reported that abiotic and biotic environments profoundly influence the distribution of animals in different habitats. The physical environment also includes everything that is not directly associated with the presence of other animals including fish (Ivoke *et al.*, 2007). The authors also reported that life patterns and activities of animals in a given ecological system are also influenced by a number of factors which could be endogenous (body size, activity, reproductive cycle pattern, nutritional status), or exogenous (hydrogen ion concentration (pH), salinity, temperature, oxygen concentration and photoperiod among others).

The pH of a solution is among the many abiotic factors that affect the survival, growth; reproduction and distribution of aquatic animals like fish (Ivoke *et al.* 2007). To support the above findings Bryan (2004) documented that the pH of surface water or water is important to aquatic life, because its affects the ability of fish and other aquatic organisms to regulate basic life-sustaining processes, primarily the exchange of respiratory gases and salts, with the water in when they live. The authors added that failure to adequately regulate these processes can result in numerous sub-lethal effects such as diminished growth rates and even in cases when ambient pH exceeds the range physiologically tolerated by aquatic organisms.

El-Sheriff and El-fekey (2009) documented that the effect of pH Levels of 6, 7, 8 and 9 on performance of Nile Tilapia (*Oreochromis niloticus*) fingerlings stocked for 60 days revealed no mortality in any of the experimental groups throughout the experimental period. They also reported that *O. niloticus* fingerlings with average weight of 19.00± 1.0g were more suitable to culture at water pH levels between 7 and 8 for optimum growth performance and survival rate than other water conditions. Bryan (2004) documented that United States Environmental Protection Agency (USEPA) concluded that a pH range of 6.5 to 9.0 provides adequate protection for the life of fresh water fish and bottom dwelling macro invertebrates. Outside this range, fish suffer adverse physiological effects that increase in severity as the degree of deviation increases until lethal levels are reached (USEPA, 1999).

The pH of cultured water of fry, fingerlings or fish may not be altered only due to left over feed and feacal samples, but often changes in lakes and streams during the day in response to photosynthetic activity (Boyd, 1990). In ponds having poorly buffered (low alkalinity) waters, the pH may fall to approximately 7 in the early morning and increase to 9 or more in the afternoon. Good fish production usually can be maintained in spite of these daily fluctuations, unless diurnal fluctuations result in ambient pH falling below 6 or being elevated above 9. These pH levels have no adverse impact on aquatic life (Bryan, 2004).

C. angullaris is also an aquaculture candidate because of its resistance to diseases, tolerance to high concentration of ammonia in water and ability to survive low concentration of oxygen in water (Bartley *et al.*, 2000 and Okechi, 2004).Information on the extent to which pH affects the survival of *Clarias anguillaris* fingerlings is still scanty and to ensure optimum exploitation of the huge animal protein, vitamins, medical, industrial and agricultural resources abound in fishes generally and *C. angullaris* species in particular. There is an urgent need to document water pH as one of the environmental requirements of this fish.

Therefore the present study was carried out to evaluate the influence of different levels of water pH on survival rates of laboratory reared *C. anguillaris* fingerlings under laboratory conditions.

Materials and Methods

Experiment and Procedures

The study was conducted at the Biology laboratory of the School of Life Sciences, Bosso Campus, Federal University of Technology, Minna. Niger State. Four hundred, 28 days old *C. anguillaris* fingerlings were purchased from a private fish farm in Lagos, Lagos state, Nigeria. The fingerlings were transported to the Biology laboratory in 50litres jerican with well aerated water through openings at the top for ventilation.

The fingerlings were acclimatized in rearing tanks for a period of seven days to allow them to recover from transportation stress and room or air temperature was maintained at 28±1^{0}C. They were also visually observed to ensure that there were no infections from the source and also to select average weight of the

fish to be cultured together (Adewolu *et al.*, 2008, Ayanwale *et al.*, 2014). During this period, the fish were fed on a commercial diet (Catco fish concentrate) by Coppens International, Holland. They were fed to satiation, morning and evening following the method of Ayanwale *et al.*, (2014). Water exchange was done twice a week in the morning. The left over feed and faecal droppings were siphoned immediately after feeding (Ghanbari *et al.*, 2012).

A Completely Randomised Design (CRD) with a total of 4 treatments, two replicates was adopted for the experiment.

Experimental Setup

The experiment consisted of 4 treatments with 2 replicates each. Treatments 1, 2, 3, and 4 had water pH of 4.0, 5.0, 6.0 and 7.30 (control) respectively. Eight plastic indoor aquaria tanks(53×35×22) cm^3 were filled with 25 litres of fresh bore hole water at 18 cm level, and each replicate was stocked with 50 fingerlings. Exchange of water from the experimental tank was done twice a week, while the removal of faecal samples, uneaten feed and monitoring of the pH of experimental tanks with the aid of pH meter were done on a daily basis, to maintain the tested pH levels (Ghanbari *et al.* 2012). The initial pH in all the experimental tanks was determined first by inserting the pH probe into the aquarium. The initial pH of the borehole water was adjusted to pH 4.0, 5.0 and 6.0 by the addition of 3% concentrated Hydrochloric acid. The stock solution of the acid was prepared by measuring 3ml concentrated HCl and 97ml of distilled water into beaker, mixed thoroughly with a clean glass rod and stored in a reagent bottle. To obtain the desired pH levels, drops of 3% concentrated HCl was added where necessary to obtain the most desire reading as displayed by the pH meter (Ivoke *et al.*, 2007). The experiment lasted for a period of six weeks.

DETERMINATION OF SOME PHYSICO-CHEMICAL PARAMETERS

Water temperatures of the treatment were determined with mercury in bulb thermometer (10-110^{0}C range). Temperature was determined by lowering the thermometer into the tanks in an inclined position for about 5 minutes to allow for equilibrium before taking the reading at 10.00 am in the morning throughout the duration of the experiment.

Dissolved Oxygen was determined by using Winkler Azide method (American Public Health Association, 1995). Water samples from the control and treatment tanks were collected by inserting 250 ml water sample bottles into the tanks and sampled water was fixed right in the laboratory with 1ml of reagent (I) (Manganous sulphate) and 1ml of reagent (II) Alkaline iodide solution (KOH + KI). About 2 ml of concentrated sulphuric acid was added to each sample and 10ml of the sample was titrated with 0.025N sodium thiosulphate using starch as indicator until it turns colourless.

Calculation was based on the formula described by Boyd (1979) as follows:-

$$\text{Dissolved Oxygen (mg/L)} = \frac{\text{Volume}(Na_2SO_3) \times \text{Normality} \times 8 \times 1000}{\text{Sample volume (ml)}}$$

Where, normality= 0.025 ml of sodium sulphite (Na_2S0_3)
8 = Equivalent weight of oxygen in water
1000 = Conversion to mg/litre

Biochemical Oxygen Demand (BOD)Water was determined by collecting water samples from the treatment tanks and were incubated for 5days in the dark before the titration for oxygen using Winkler Azide method (APHA, 1995).

BOD 5 mg/L = Dissolved oxygen at day 1 – Dissolved oxygen at day.

The pH of the water samples from the control were determined with Jenway 3305 pH meter model at room temperature. The pH meter probe was inserted into the sampled water for about 5 minutes until it stabilized before the reading was taken. The meter was standardized with buffer solutions of pH 4.0, 7.0 and 9.0 before the readings were taken.

Ammonia (NH_3) level of water was determined by pipetting 100ml of the water samples from each of the experimental treatment tanks into a Markham distillation apparatus (Kjeldal flask) and there after 5ml of 40% NaOH was added. The flask was connected to the condenser and the cooling water was turned on. About 10ml of 40% boric acid (H_3BO_3) solution was placed under the condenser ensuring that the tip of the condenser was immersed in the receiving solution and distilled slowly until 50ml of the distillate was collected in the receiving flask. The ammonia

was determined from the distillate by titrating with 0.01M HCl until the colour at the end point changed from green to pink (APHA, 1995). Calculation was based on the formula below

$$NH_3(mg/L) = \frac{\text{Titre value} \times 14 \times 0.01 \times 1000}{V}$$

Where 0.01 = molarity of HCl used as titrant; 14 is the molecular mass of nitrogen; 1000 is the conversion to mg /litre and V is the volume of sample used.

The manual electrical conductivity metre (CD 4303 Lutron) probe was inserted into the sampled water for about 5 minutes until it stabilizes before the reading was taken. The readings were expressed in microseimen (µs/cm).

Data Analysis of the Experiment

The data collected were analyzed for significant differences ($P < 0.05$) by the analysis of variance (ANOVA) using a Computer Statistical Package for Social Sciences (SPSS). Duncan Multiple Range Test (Duncan, 1955) method was used to separate the means where there were statistically significant differences ($P < 0.05$).

Results

The results of the weekly survival rates of *C. anguillaris* fingerlings cultured in different pH levels for a period of six (6) weeks is presented in Table 1.The weekly survival rates ranged from 49.50±0.50 at pH 7.3 to 50.00± 0.00% at pH 6.0 of *C. anguillaris* fingerling were not significantly different ($p > 0.05$) among treatments at the end of the study. Interestingly, the weekly survival rates of the fingerlings cultured in pH 5.0, 6.0 and 7.3 were not significantly different ($p > 0.05$) in weeks 1, 2, 5 and 6 respectively.

Table 1: Mean Standard deviation of weekly survival rates of laboratory reared *Clarias anguillaris* cultured in different pH levels for a period of six weeks.

	Week					
Treatment	1	2	3	4	5	6
4.0	43.50 + 0.50^a	34.50 I 0.50^a	34.50±0.50^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^c
5.0	50.00±0.00^b	48.00 I 1.60^b	47.00±0.00^b	45.00+0.00^b	45.00±0.05	45.00±0.00^b
6.0	50.00±0.00^b	50.00+0.00^b	50.00±0.00^c	50.00±0.00^c	50.00±0.00^b	50.00±0.00^b
7.3 (control)	49.50±0.50^b	49.50±0.50^b	49.50±0.50^c	49.50±0.50^c	49.50±0.50^b	49.50±0.50^b

Values are Means ± standard deviation, values followed by the same superscript(s), in the same column, are not significantly different at ($P > 0.05$) tested by DMRT.

The results of some physicochemical parameters of cultured water of *C. anguillaris* fingerlings reared in different pH levels for a period of six weeks is depicted in Table 2. The dissolved oxygen concentration (2.67±0.00 mg/L) of water media of *C. anguillaris* fingerlings reared in pH 4 was significantly lowest ($P < 0.05$) at the end of the study. However, water temperature ranged from 28.21 ± 0.12 to 28.23 ± 0.03°C), electrical conductivity ranged from 508.50 ± 13.00 in pH 4.0 to 547.83±10.50 µs/cm at pH 6.0 and ammonia concentration ranged from 1.69± 0.04 in pH 4.0 to 1.85±0.21 Mg/L in pH 7.13 of the cultured water of *C. anguillaris* fingerlings were not significantly different ($P > 0.05$) at the end of the study. But, the BOD (2.50±0.17 Mg/L) of the cultured media of *C. anguillaris* fingerlings reared in pH 4.0 was significantly higher ($P < 0.05$) when compared with other treatments at the end of the study.

Table 2: Mean Standard Deviation of Physico Chemical Parameters Measured during the Experiment on Effects of Different Levels of Water pH on the Survival Rates of Laboratory reared *Clarias anguillaris* Fingerlings.

Treatments	Dissolved Oxygen Mg/L	Biochemical Oxygen Demand (Mg/l)	Electrical Conductivity µs/cm	Ammonia (Mg/L)	Temperature (°C)
4.0	2.67 ± 0.00[a]	2.50± 0.17[b]	508.50±13.00[a]	1.69±0.04[a]	28.21±0.12[a]
5.0	8.05±0.22[b]	1.55±0.45[a]	528.67±14.58[a]	1.75±0.16[a]	28.23±0.03[a]
6.0	8.55±0.62[b]	2.03±0.53[a]	547.83±10.50[a]	1.80±0.13[a]	28.23±0.06[a]
7.3 (control)	7.42±0.08[a]	1.58±0.58[a]	528.17 I ±12.33[a]	1.85±0.21[a]	28.21±0.12[a]

Values are means standard deviation, Values felling by the same superscript(s) in the same 'column, are net significantly different at (P

Discussion

The study revealed that water pH levels of 6.0 to 7.3 (control) could support the survival of *C. anguillaris* fingerlings under laboratory conditions. This finding could be due to the ability of the fingerlings to regulate basic life-sustaining processes, primarily the exchange of respiratory gases and salts, in which they live. The above finding was in conformity with the works of El-sheriff and El-fekey (2009) who documented that Nile Tilapia (*O. niloticus*) cultured at pH levels of 6.0, 7.0 8.0, 9.0 for a period of 60 days recorded no mortality.

The consistent reduction in the percentage weekly survival rates of fingerlings from week 1 to 6 cultured in pH 4.0 may be attributed to toxic action of hydrogen ions on cat fish (*C. anguillaris*) under pH4 condition. This acidic medium involved the production of mucus on the gill epithelium which interferes with the exchange of respiratory gases, ions exchange across the gill, and or acidosis of the blood which affect oxygen uptake (Boyd, 1990).

This finding was also in conformity with the works of Gaunder (2005) who observed that the acid and alkaline death points for fish are about pH4 and 11 respectively with reproduction and growth diminishing with increasing acidity or alkalinity. The survival of *C. anguillaris* fingerlings cultured in pH 5.0, 6.0 and 7.3 (control) under captivity in weeks 1,2,5 and 6 respectively suggests that the fingerlings were capable of regulating their metabolic modulations and physiological functions through acclimation process to compensate the acidic stress imposed by the external media (Srineetha *et al.*, 2014). The authors also added that this could be the major reason for the successful survival of fry fingerlings in acidifying water. The significant decrease in the dissolved oxygen in pH4.0 might be due to hypoxic condition that prevailed in the external media (Srineetha *et al.*, 2014).

They also reported that it could be due to reduced gases exchange through gill surface because of excessive formation of mucus and also reduced oxygen carrying capacity of the blood due to altered blood pH on exposure to acidic media.

The findings of this study also revealed that water pH levels had no influence on the water temperature of the cultured media of *C. anguillaris* fingerlings. But the values were within the range of 26.00 -32.00°C documented for fish culture in the tropics (Ayanwale *et al.*, 2014). Similarly, pH had no influences on the ammonia concentration of the cultured media of *C. anguillaris* fingerlings. This may be due to constant exchange of water, siphon of uneaten feed and faecal samples, thus preventing accumulation of organic load (Ghanbari *et al.*, 2012).

Water pH levels investigated had no effect on the electrical conductivity but the values obtained were higher than the range from < 25 to> 500 us/cm documented for pond fish culture (Federal Environmental Protection Agency FEPA, 1991). The electrical conductivities values obtained from the treatments in this study suggest higher levels of dissolved solids which signify presence of pollutants in the cultured media of *C. anguillaris* fingerlings. This may be responsible for the lower values recorded in the weekly percentage survival rates of fingerlings during the experimental period (Keremah *et al.*, 2014).

The higher value of BOD recorded in the fingerlings cultured in pH4.0 confirms the

reduction in the DO concentration in the media and this could result in fingerlings being stressed, suffocated and resulted into fish kills (Keremah *et al.*, 2014).Although growth parameters were not monitored during this study, the pH range 6.0 to 7.3 could be deduced to had supported the growth of *C. anguillaris* fingerlings. This is because the pH range was within the documented range of 6.5 to 9.0 which provide adequate protection for freshwater fish (Bryan, 2004).

Conclusion

The *Clarias anguillaris* fingerlings cultured in pH 4.0 had the lowest weekly survival rates. The optimum pH range 6.0 to 7.3 (control) will support the survival of *C. anguillaris* fingerlings. Water pH had no influence on the water temperature, ammonia concentration and electrical conductivity. The dissolved oxygen concentration and biochemical oxygen demand of *C. anguillaris* fingerlings cultured in pH 4 were significantly reduced at the end of the study.

Recommendation

It is therefore recommended that further studies should be done to confirm the predicted pH range (6.0 to 7.3) on the growth parameters of *C. anguillaris* fingerlings.

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