Effect of pH on Hatching Success and Larval Survival of African Catfish *(Clarias gariepinus)*

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Abstract: A study was carried out to determine the effect of pH on hatching success and larval survival of *Clarias gariepinus* which is the dominant fresh water fish produced in Nigeria. The incubation time, hatching rate and larval survival of *Clarias gariepinus* were compared at pH levels between 4.0 and 10.0. The incubation time extended from 17 hours at pH 6.5. – 8.5 to 20 hours at pH 4.5 and 9.5. No hatch occurred at pH 4.0 and 10.0. Mean hatching rate increased from 31.18% at pH 4.5 to 69.84% at pH 8.0 and then declined to 34.21% at pH 9.5. Larval activity depressed at low and high pH whereas larvae were very active at pH 7.5-8.5. The results indicate that the optimum pH range for normal hatching and larval survival of *Clarias gariepinus* is pH 7.5-8.5.
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1. Introduction

There is rapid development in technology for the culture of the African catfish *Clarias gariepinus* (Hecht *et al*., 1988) which ranks high on the consumer preference list for fresh water fish species in quite a number of countries in Africa (Huisman, 1986). It has also been reported as the dominant freshwater fish produced in Nigeria (Rehman, 1980). The problem at present is inadequate supply of fish seed to fish farmers due to large-scale mortalities of fish that occur in the early life stages. An ideal hatchery system with optimal water quality has not yet been developed in Nigeria. It has been reported that development problems may often be induced by unfavourable abiotic environmental factors and that conditions considered optimal for older fish may be limiting to eggs and successive developmental stages (Blaxter, 1974; Adebayo *et al*., 2007).

The effects of low pH on fish has been extensively studied and many reviews are available (Haines, 1981; Dillon *et al*., 1984; Jordahl, 1984; Jordahl and Benson, 1987). In studies where the effects of acid have been investigated throughout the whole life cycle (Menendez, 1976; Craig and Baksi, 1977) eggs and larval stages have been found to be the most vulnerable stages. Furthermore, acidification has been reported to decrease hatchability (Trojnar, 1977), and reductions in egg viability (Menendez, 1976; Beamish, 1976).

The effects of temperature (Ajuzie and Appelbaum, 1996), salinity (Oladosu *et al*., 1999), water hardness (Molokwu and Okpokwasili, 2002a) and bacterial load (Ariole and Okpokwasili, 2012) on the embroyonic development and larval survival of *Clarias gariepinus* have been reported. The effect of fertilizer effluent (Ekwezor *et al*. ,2001) and heavy metal content (Obasoham and Oronsaye, 2000) of adult *Clarias gariepinus* have been established. The effect of different diets on the growth and survival of larvae of *Clarias albopunctatus* (Aguigwo, 1999) and catfish hybrid hatchlings (Ezechi and Nwuba, 2007) has been studied. Studies of comparative growth performance of *Clarias anguillaris* and its hybrid-heteroclarias have been reported (Aguigwo, 1998). The microbial flora of *Clarias gariepinus* in the early stages of development has been established (Molokwu and Okpokwsili, 2002b). Data on the optimal pH range for *Clarias gariepinus* survival and viability is not available. Such Information is pertinent if the best incubation condition for *Clarias* eggs is to be realized. Therefore, this study was carried out to investigate the effects of pH on incubation time, hatching rate and larval survival of *Clarias gariepinus* as part of a longer-term investigation into the influence of water quality on hatching success and survival of this tropical fish.

2. Materials and Methods

The eggs of *Clarias gariepinus* were obtained at African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt by artificial breeding of brood–fish as described by Delince *et al*. (1987). The fertilized eggs were distributed into 5 1 circular plastic tanks (30 cm diameter) containing aged tap water adjusted to various pH levels (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0) using 2M HCl and 2M NaOH. The pH measurements were

carried out using Jenway pH meter (Model 3015). Calibrations were performed with buffers of pH 4.0, 7.0 and 9.0. Each pH level was prepared in three replicates.

The fertilized eggs in each tank were counted and the number noted. Dead eggs and larvae were removed with forceps. The duration of incubation, in hours, was recorded for each pH. The hatched eggs were counted and the hatching percentage was determined for each pH. Deformed larvae were also counted and their percentage was determined based on the total hatched larvae.

Incubation remnants, dead larvae and waste matter were siphoned off every day during larval rearing to avoid any form of stress and 50% of water in each tank was replaced into the water of same pH. Three developmental periods were defined; i.e., the egg period, the hatching period and the yolk–sac period. The egg period began from the time of placement of eggs for incubation and ended when the eggs began to hatch. The hatching period began when the first eggs hatched and yolk- sac period extended from the end of the hatching period until the yolk-sacs of the fry were absorbed (yolk-sac absorption was determined visually). The percentage survivals of eggs and larvae at the end of each developmental period were determined.

An arcsine transformation on percentage survival was used to meet ANOVA assumptions (Sokal and Rohlf, 1981). Data on hatchability and larval survival were analysed with the analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT). Significance was established at the 0.05 level.

3. Results

The effects of pH on incubation period, hatchability and egg and larval survival of *Clarias gariepinus* are presented in Figures 1 and 2 and Table 1. The incubation time extended from 17 hours at pH 6.5-8.5 to 20 hours at pH 4.5 and 9.5 (Figure 1).

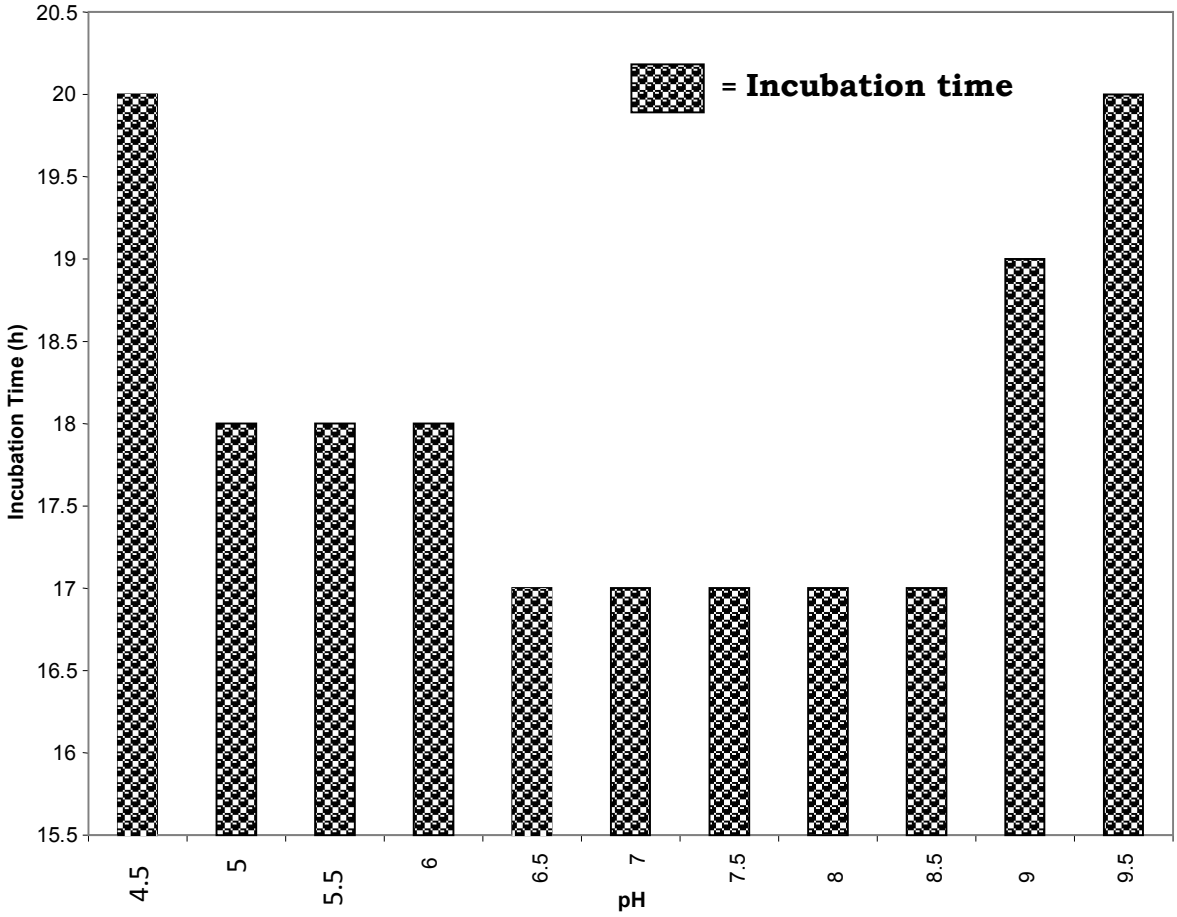

Figure 1. Incubation time to hatching of *Clarias gariepinus* eggs exposed to different pH values

Mean hatching rate increased from 31.18% at pH 4.5 to 69.84 at pH 8.0 and then declined to 34.21% at pH 9.5 (Figure 2). Figure 2 also showed that there were no deformities at pH 6.5-8.5. Deformities were observed beyond this range and increased with increase in acidity and alkalinity.

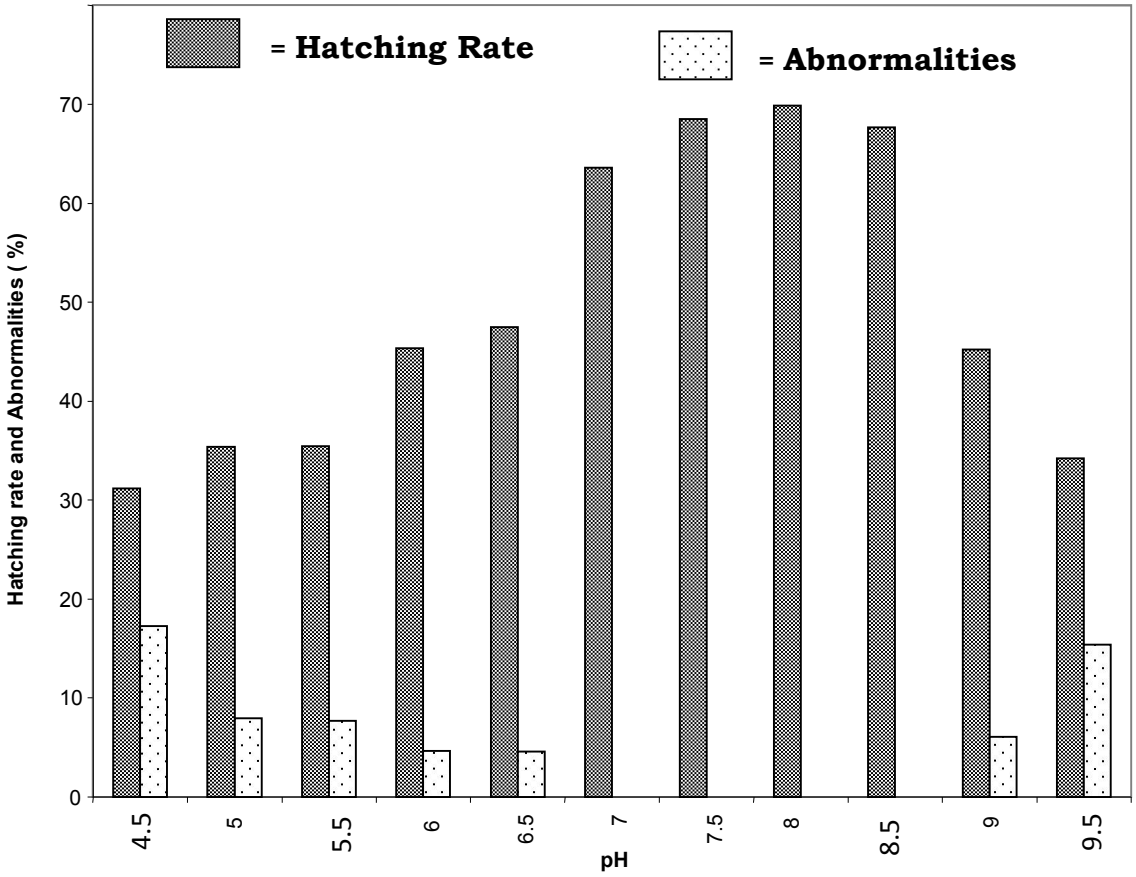

Figure 2. Hatching rate of fertilized eggs and percentage abnormalities of *Clarias gariepinus* lavae *(*or hatchlings*)* exposed to different pH values.

Table 1. Survival (Mean ±SD) of *Clarias gariepinus* eggs and larvae exposed to different pH values

	Egg Period			Hatching period		Yolk sac period		
pH	**Initial number of eggs**	**Survivors to hatching period**	**Egg survival (%)**	**Survivors to end of hatching period**	**Survival from end of egg period (%)**	**Survivors to end of yolk sac period**	**Survival from end of hatching period (%)**	**Survival from end of egg period (%)**
4.0	171	0	0					
4.5*	93	29	31.18^{z}± 0.57	9	31.03±0.70	0		
5.0*	178	63	35.39^{x} ± 2.00	41	65.08±0.72	0	8.33±27	
5.5	110	39	35.46^{x} ± 1.74	36	92.30±0.50	3	31.71±1.4	7.69^{Y}± 1.56
6.0	89	43	48.32^{d} ± 1.70	41	95.35±1.1	13	46.30±1.5	30.23^{d}±1.09
6.5	120	57	47.50^{d}± 0371	54	94.74±1.2	25	29.89±1.07	43.86^{c}±1.30
7.0	140	89	63.57^{c} ± 075	87	97.75±1.9	26	87.50±2.00	29.21^{e}±0.35
7.5	108	74	68.82^{a} ± 0.37	72	97.30±1.3	63	81.18±0.5	85.14^{a}±1.36
8.0	126	88	69.84^{b}± 0.10	85	96.59±0.9	69	87.5±0.35	78.41^{b}±0.67
8.5	133	90	67.67^{a} ± 0.28	88	97.78±0.7	77	72.00±0.80	85.56^{a}±0.95
9.0	146	66	45.21^{e} ± 1.00	25	37.88±0.6	18		27.27^{x}±0.51
9.5*	7.6	26	34.21^{y}±1.71	6	23.08±0.54	0		
10.0	99	0	0					

No eggs hatched at pH 4.0 and 10.0

* All yolk sac larvae at these pH died before the end of yolk sac period. Mean values within each column which do not have the same superscript letter are significantly different ($p < 0.05$).

Table 1 revealed that no eggs survived (hatched) at pH 4 and 10 and that survival was higher during the hatching period at pH 5.5-8.5 than during the egg and yolk sac periods. All yolk sac larvae at pH 4.5, 5.0 and 9.5 died before the end of yolk-sac period. Significant differences were observed in hatching rates and also in larval survival at the different pH values (p=0.05). However, there was no significant difference in hatching rate and also in larval survival at pH 7.5 and 8.5. Survival at the end of yolk sac period was high at pH 7.5 and 8.5 (85 – 86%), still high at pH 8.0 (78.41%), moderate at pH 6.5 (43.86%) and low at pH 4.5-6.0, 7.0, 9.0 and 9.5 (0-30%; Table 1).

4. Discussion

The pH had a pronounced effect on the time required for development and hatching of *Clarias gariepinus*. This was observed when the incubation time extended from 17 hours at pH 4.5 and 9.5 (Figure 1) and no hatching occurred at pH 4 and 10 (Table 1).

Other workers variably reported their findings on incubation time in acid waters for various fishes. Peterson *et al*. (1980) reported that hatching time of Atlantic salmon (*Salmo solar*) eggs was independent of pH down to 4.5 if they were reared from fertilization in acid water, but hatching was delayed if they were transferred from neutral pH water after the eyed stage. A delayed hatching of eggs at low pH was also found in the zebra fish (*Brachydanio rerio*) by Johansson *et al*. (1973), in perch (*Perca fluviatilis*) by Runn *et al*.(1978) and in brook trout (*Salvelinus fontinalis*) by Swarts *et al*. (1978). However, eggs of *Salvelinus fontinalis* have also been found by Trojnar (1977) to have an earlier hatching at low pH and by Menendez (1976) to have an incubation time unaffected by pH. The results of Carrick (1979) also show no relationship between pH and the onset of hatching of the eggs of various Salmonids including brown trout.

Survival of *Clarias gariepinus* eggs and larvae was related to pH with lowest surviving occurring in the low acidic and high alkaline pH and highest in the neutral and slightly acidic and alkaline pH (Table 1 and Figure 2). The low survival rate during the developmental periods at low acidic and high alkaline pH indicated that the eggs and the newly hatched *Clarias gariepinus* larvae were sensitive to low and high pH. The sensitivity of fish larvae to low pH has been demonstrated in the laboratory studies with brook trout (Menendez, 1976; Trojnar, 1977) and in field studies with other salmonids (Huisman *et al*. 1983; Lacroix *et al*. 1985). Both the activity of the hatching enzyme (chorinase) and of the larvae are inhibited at low pH (Haya and Wainwood, 1981; Peterson and Martin – Robichaud, 1983), thus delaying or inhibiting hatching. The deformities which were observed beyond 6.5-8.5 (Figure 2) may be due to the spinal damage of the larvae. Spinal flexures of the larvae appeared to be a common response to various environmental stresses during ontogenic development (Onuoha and Nwadukwe, 1990).

Results of the analysis of various and Duncan's multiple range test (p= 0.05) indicated that there was no significant differences in the hatching rate at pH 7.5 and 8.5 and that this hatching rate was lower than the highest hatching rate of 69.85% recorded at pH 8.0 (Table 2). Statistical analysis also revealed that larval survival at pH 7.5 was not significantly different from that at 8.5 (where the highest larval survival of 85.56% was obtained but was significantly higher than that recorded at pH 8.0. It was also observed that larval activity was depressed at low and high pH whereas larvae were very active at pH 7.5-8.5 (Table 2). The results indicate that the optimum pH range for normal hatching and larval survival of *Clarias gariepinus* is pH 7.5-8.5. Jordahl and Benson (1987) in their study on the effect of low pH on survival of book trout embryos and yolk sac larvae in West Virginia streams reported that survival rates for all developmental stages, from embryo to yolk sac larvae, were highest at Roaring creek (pH 6.1-7.2) and lowest at little Laurel creek (pH 5.0).

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