Effects of Inorganic Nutrients on Survival of Eggs and Larvae of *Clarias gariepinus*

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Abstract: The effects of inorganic nutrients (nitrate, sulphate and phosphate) on survival of eggs and larvae of *Clarias gariepinus* from fertilization to complete yolk absorption were investigated. Fertilized eggs were incubated at fourteen levels of nitrate ranging from 0.02 – 700mg/L, sixteen levels of sulphate ranging from 0-1000mg/L and ten levels of phosphate ranging from 0-500mg/L. The hatching rate was highest (73.24%) at nitrate concentration of 70mg/L while larval survival was highest (26.09%) at nitrate concentration of 150mg/L. The hatching rates of the eggs exposed to 70-150mg/L were significantly higher than those obtained from other nitrate concentrations including dilution water (0.02mg/L NO_3^-N). The larvae exposed to nitrate concentrations other than this range (70-150mg/L) died before the end of yolk sac period. Hatching rate was highest 80-81% at sulphate concentrations of 100-120mg/L while larval survival was highest (63.33%) at sulphate concentrations of 100mg/L. The sulphate concentrations other than 50-130mg/L had zero percent survival. Phosphate concentrations up to 80mg/L had no effect on incubation time. Abnormalities in hatchlings were observed beyond 20mg/L and increased with increase in phosphate concentration. No eggs hatched at 500mg/L and the highest larval survival of 52.17% was obtained at 20mg/L phosphate. These data suggest that maximum normal hatching and larval survival of *Clarias gariepinus* may be anticipated if the concentration of nitrate is within the range of 70-150mg/L and sulphate is about 100mg/L. A phosphate concentration within 5-20mg/L is recommended for optimal normal hatching and high viability of *Clarias gariepinus* eggs.
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1. Introduction

The African catfish, *Clarias gariepinus*, is rapidly gaining status as prime aquaculture species in a number of African, Asian and European countries (Ariole and Okpokwasili, 2012a). In Nigeria, it is in high market demand as table fish, being tasty and scaleless. Like many other organisms early developmental stages of *Clarias gariepinus* are probably the most sensitive stages of its life cycle. Developmental problems may often be induced by unfavourable abiotic environmental factors (Adebayo *et al*., 2007).

The effects of water hardness (Molokwu and Okpokwasili, 2002a), pH (Ariole and Okpokwasili, 2012b) and indigenous probiotics (Ariole and Okpokwasili, 2012c) on the embryonic development and larval survival of *Clarias gariepinus* have been reported. The microbial flora status in *Clarias gariepinus* hatchery systems has been established (Molokwu and Okpokwasili, 2002b). No work has yet been published on the effects of inorganic nutrients such as nitrate, sulphate and phosphate on the developmental biology of *Clarias gariepinus.*

A thorough understanding of the responsible ecological factors such as nutrients and their specific limiting ranges is desirable to maintain optimal rearing conditions (Ariole and Okpokwasili, 2012b). Nutrients are of particular interest because due to increased agricultural and industrial activities, there is an increasing evidence of groundwater pollution by these nutrients (Prakasa Rao and Puttanna, 2000) and borehole water is the water source for most Nigerian fish hatcheries. Furthermore, industrial waste water, household waste water, runoff from a hazardous waste site or naturally decaying material can put these nutrients into waterways, rivers, lakes and streams (Rutkoviene *et al*., 2005). These nutrients will enhance the growth of aquatic vegetation or phytoplankton and algal blooms disrupts normal functioning of the ecosystem, causing a variety of problems such as a lack of oxygen needed for fish and shellfish to survive. Eggs and larvae of fish, like all other higher organisms are particularly susceptible to toxic substance (Köprücü and Aydin, 2004).

Destruction at this stage of development has fast effect on the size of fish population although adult fish may be tolerant to a certain level of toxicity. The number of spawned eggs and their survival rate are the most common indicators of the long-term impact of pollutants on fish reproduction (Pandey, 2000). The hatchability of eggs and survival of larvae from exposed parents may reflect both pollutant induced change in eggs structure which decrease fertilization rate and tetralogial effects resulting in deformed embryos (Köprücü and Aydin, 2004). The survival of eggs, larvae and fry which are

particularly vulnerable to pollutants have a major impact on population dynamics (Adebayo, 2004).

Therefore, this study was conducted to determine the effect of nitrate, sulphate and phosphate on *Clarias gariepinus* from fertilization to complete yolk absorption. This knowledge would help improve management of tropical fish hatcheries.

2. Materials and Methods

The eggs of *Clarias gariepinus* were obtained from the hatchery at the African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt by artificial breeding of the brood fish (Ariole and Okpokwasili, 2012c). Final maturation followed by ovulation was induced in female spawners with hormone treatment. The riped eggs were procured by stripping and milt was procured by dissection of a male donor. Few drops of milt solution were added onto the eggs and the sexual products mixed by gentle shaking of the bowl.

The fertilized eggs were distributed into 5L circular plastic tank (30cm in diameter) containing different nitrate concentrations. Nitrate solutions were made by dilutions of a stock solution of sodium nitrate ($NaNO_3$) to final concentrations ranging from 0.05 to 700mg/L. Each concentration had three replicates. The fertilized eggs were also distributed into 5L circular plastic tank (30cm in diameter) containing water at different sulphate concentrations. Sulphate solutions were made by dilutions of a stock solution of sodium sulphate (Na_2SO_4) to final concentration ranging from 5-1000mg/L. Each concentration had three replicates. The fertilized eggs were, again, also distributed into 5L circular plastic tank (30cm in diameter) containing different concentrations of phosphate. Phosphate solutions were made by dilutions of a stock solution of potassium dihydrogen orthophosphate (KH_2PO_4) to final concentrations ranging from 5 to 500mg/L. The dilution water was used as control. Each concentration had three replicates.

The tap water base (dilution water) analyzed according to the method given by APHA (1998) had a pH of 6.5, total alkalinity of 9.0mg/L, total hardness of 10mg/L, sulphate concentration of 0.00mg/L, nitrate concentration of 0.02mg/L and phosphate concentration of 0.00mg/L. No phenolphthalein alkalinity was found. The initial number of fertilized eggs in each tank was noted (Tables 1, 3 and 5). The dead eggs and larvae were removed with forceps and the percentage survival calculated (Tables 1, 3, and 5). The duration of incubation time (from fertilization to first hatch) was recorded for each nitrate and sulphate concentration. The hatched eggs were counted and the percentage determined based on the total hatched larvae.

During larval rearing, incubation remnants, dead larvae and waste matter were siphoned off everyday to avoid any form of stress and 50% of the water in each tank was replaced. Three developmental periods were defined - the egg period, the hatching period and the yolk-sac period. The egg period began from the time of placement and ended when the eggs began to hatch. The hatching period began when the first eggs hatched and ended when all eggs had hatched. The 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 survival of eggs and larvae at the end of each developmental period were determined.

2.1. Statistical analysis

The percentage hatchability and larval survival were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to determine differences ($p<0.05$) between tested groups. All statistics were performed using SPSS 10 for Windows.

3. Results Analysis

The incubation time increased from 17 hours at nitrate concentrations of 0.02-0.05mg/L to 22 hours at nitrate concentrations of 200-700mg/L (Figure 1).

The mean hatching rate decreased from 37.80% at nitrate concentration of 0.02mg/L to 15.00% at 0.5mg/L and then increased to 73.24% at 70mg/L and thereafter declined to 20.37% at nitrate concentration of 700mg/L (Figure 2). Abnormalities in the larvae were observed beyond 150mg/L and increased with increase in nitrate concentration (Figure 2).

The effect of nitrate on egg and larval survival presented in Table 1 revealed that the hatching rates of the eggs exposed to 70-150mg/L were significantly higher than those obtained from other nitrate concentrations including dilution water (0.02mg/L NO_3^-. N).

Furthermore, the larvae exposed to nitrate concentrations other than this range (70-150mg/L) died before the end of yolk sac period. Highly significant differences were observed in hatching rates and also in larval survival at the different nitrate concentrations ($p<0.05$) (Table 2). Hatching rate was highest (73.24%) at 70mg/L while larval survival was highest (26.09%) at 150mg/L. These data suggest that maximum normal hatching and larval survival of *Clarias gariepinus* may be anticipated if the concentration of nitrate is within the range of 70-150mg/L.

The incubation time increased from 17 hours at sulphate concentrations of 0-20mg/L to 22 hours at sulphate concentrations of 300-1000mg/L (Figure 3).

Abnormalities in the larvae were observed beyond 170mg/L and increase with increase in sulphate concentration (Figure 4).

Table 3 revealed that the larvae exposed to sulphate concentrations other than 50-130mg/L had zero percent survival and that the highest larval survival of 63.33% was recorded at 100mg/L.

The hatching rates of 80.36% and 80.90% recorded at 100mg/L and 120mg/L respectively were not significantly different, but were significantly higher than other hatching rates ($p < 0.05$) (Table 4). These results indicate that the optimum sulphate concentration for normal hatching and larval survival of *Clarias gariepinus* is about 100mg/L.

The effects of water soluble phosphate on incubation time and hatching rate presented in Figures 5 and 6 respectively revealed that phosphate concentrations of up to 80mg/L had no effect on incubation time but rather a pronounced effect on hatching rate was evident. Abnormalities in larvae were observed beyond 20mg/L and increased with increase in phosphate concentration with a maximum of 50% at 320mg/L (Figure 6).Moreover, no eggs hatched at 500mg/L and the highest larval survival of 52.17% was obtained at 20mg/L phosphate (Table 5).

Results of the analysis of variance and Duncan's multiple range test ($p < 0.05$) indicated that there were no significant difference in hatchability at 5, 10 and 20mg/L phosphate and that the hatching rate at these concentrations were significantly higher than hatchability at 0, 240 and 320mg/L (Table 6). Furthermore, there were significant differences in larval survival with the peak of 52.17% at 20mg/L phosphate (Table 6). These observations suggest that the optimal normal hatching and larval survival of *Clarias gariepinus* may be expected if the concentration of water soluble phosphate is within 5-20mg/L.

4. Discussion

The results of the effect of nitrate on survival of eggs and larvae of *Clarias gariepinus* shown in Figures 1 and 2 and Tables 1 and 2 imply that nitrate affects the suitability of water for use in *Clarias gariepinus* hatchery and that *Clarias* eggs and yolk sac larvae are sensitive to high levels of nitrate beyond 150mg/L. It has been reported that nitrate-nitrogen levels of below 90mg/L seem to have no effect on warm-water fish, but salmon and other cold-water fishes are more sensitive (EPA, 2002).

The results of the effect of sulphate on survival of eggs and larvae of *Clarias gariepinus* shown in Figures 3 and 4 and Tables 3 and 4 imply that sulphate affects the suitability of water for use in *Clarias gariepinus* hatchery and that *Clarias* eggs and larvae are sensitive to high levels of sulphate beyond 100mg/L. To protect freshwater organisms in British Columbia, water guideline of 100mg/L maximum for dissolved sulphate, measured as SO_4, is recommended (EPD, 2000).

These results of the effect of water soluble phosphate on survival of eggs and larvae of *Clarias gariepinus* shown in Figures 5 and 6 and Tables 5 and 6 imply that *Clarias gariepinus* eggs and larvae are sensitive to high levels of phosphate and low levels of phosphate enhanced egg and larval survival. The deformed larvae were incapable of normal locomotion and survival. The deformities developed as a result of interference of phosphate in cell differentiation and morphogenesis. This effect had been demonstrated for some pesticides in Common carp (Köprücü and Aydin, 2004). High levels of phosphate are toxic to fish (Halvorson and Smolowitz, 2009) and pollutants are known to affect the metabolism of carp (*Cyprinus carpio*) at certain stages of development (Köprücü and Aydin, 2004).

All three inorganic nutrients investigated in the present study namely nitrate, sulphate and phosphate enhanced egg hatchability and larval viability of *Clarias gariepinus* at concentrations higher than the dilution water. It is possible that food reserve in the yolk was exhausted during hatching and the developing embryos perhaps utilized the nutrients from the external water medium. The uptake was presumably mediated through the permeable cell membrane by a cationic exchange mechanism. Incubation of eggs below or above the range optimal for hatching and rearing may result in malformation of the embryo. Molokwu and Okpokwasili, 2002a stated that if the incubating medium, for fresh water fish egg, has a lower ionic concentration (hypo-osmotic) than the egg, premature bursting of the egg from excessive water absorption may occur and that a hyper-osmotic medium will prevent proper swelling of the egg or even dehydrate and shrink the egg causing spinal damage to the larvae. This may be why deformities (because of spinal damage of the larvae) were observed beyond 150mg/L $NaNO_3$, 170mg/L Na_2SO_4 and 20mg/L KH_2PO_4 in this study. Spinal flexures of the larvae appeared to be a common response to various environmental stresses during ontogenic development (Molokwu and Okpokwasili, 2002a).

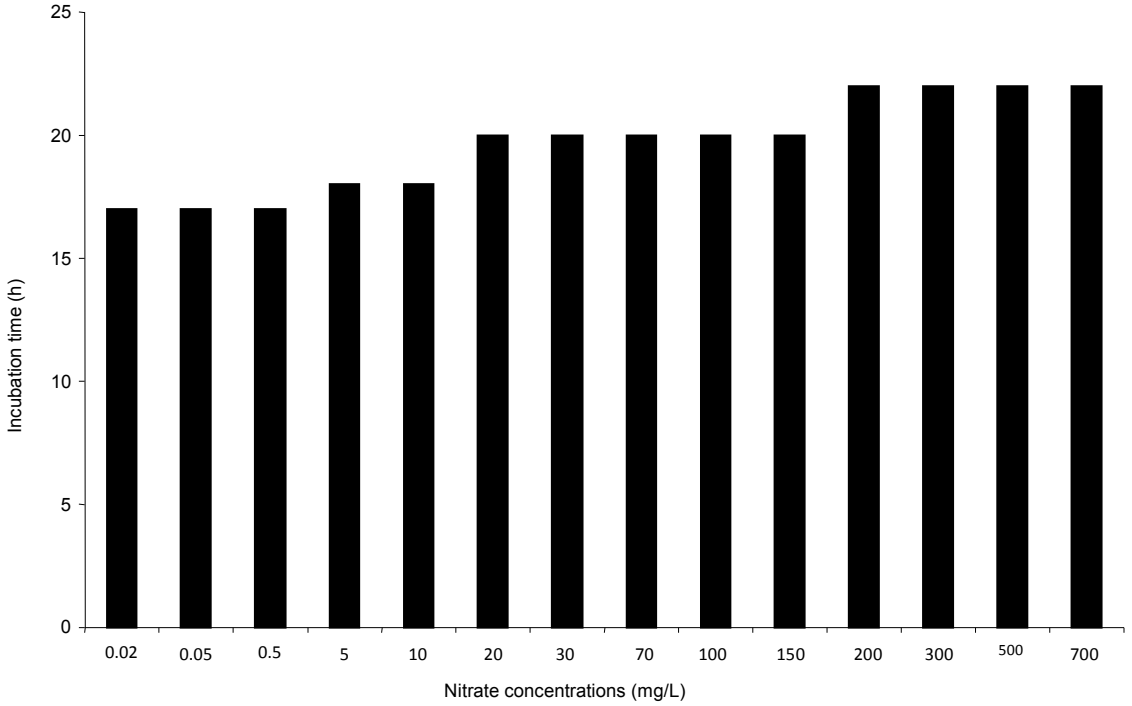

Figure 1: Incubation time to hatching of *Clarias gariepinus* eggs exposed to different nitrate concentrations

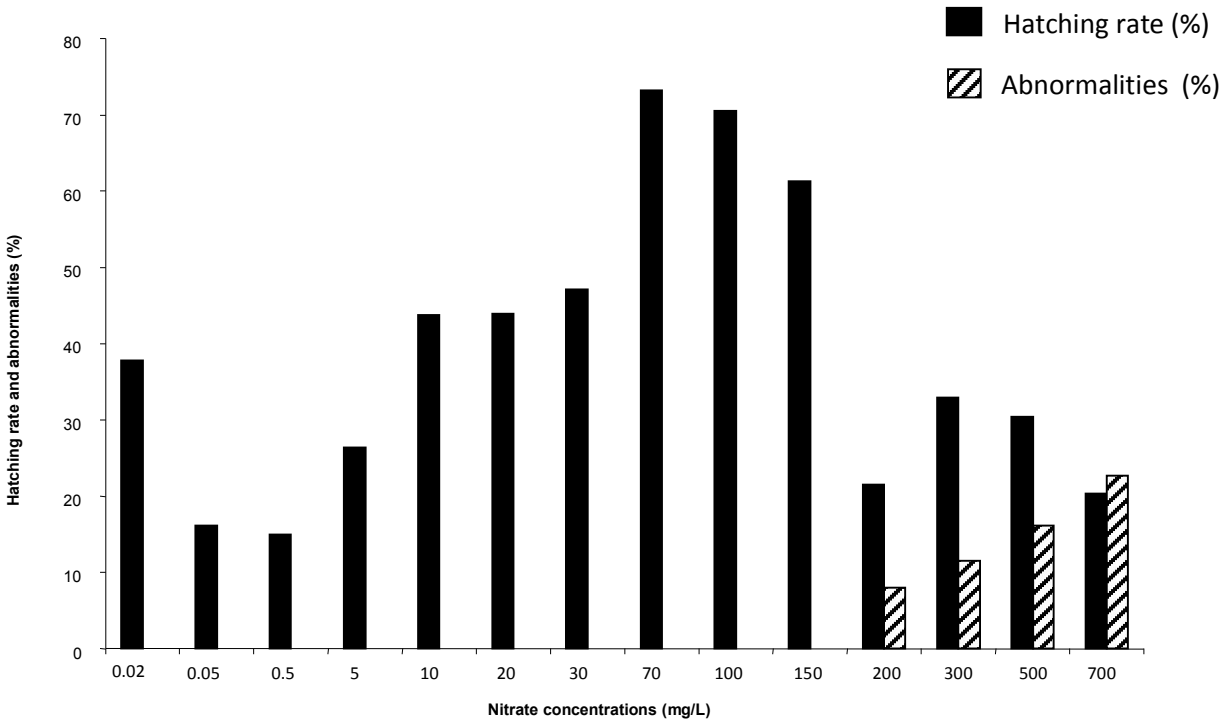

Figure 2: Hatching rate and percentage abnormalities of *Clarias gariepinus* eggs exposed to different nitrate concentrations

Table 1: Survival of *Clarias gariepinus* eggs and larvae exposed to different nitrate concentrations

Nitrate concentration (mg/L)	Egg period			Hatching period		Yolk sac period		
	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 (%)
0.02*	127	48	37.80±0.42	42	87.5±0.46			
0.05*	105	17	16.19±0.24	13	76.47±0.57			
0.5*	180	27	15.00±0.18	15	55.56±0.31			
5*	91	24	26.37±0.71	19	79.17±0.21			
10*	96	42	43.75±0.64	40	95.24±1.03			
20*	107	47	43.93±0.10	44	93.62±0.99			
30*	53	25	47.17±0.33	24	96.00±1.10			
70	71	52	73.24±0.83	52	100.00±0.0	10	19.23±0.53	18.96±0.68
100	78	55	70.51±0.58	53	96.36±0.63	10	18.87±1.12	18.18±0.76
150	75	46	61.33±0.14	46	100.00±0.0	12	26.09±1.02	26.09±0.23
200*	116	25	21.55±0.72	20	80.00±0.34			
300*	79	26	32.91±0.72	22	84.62±0.45			
500*	102	31	30.39±0.86	22	70.79±0.59			
700*	108	22	20.37±1.94	20	90.91±0.83			

*All yolk sac larva at these concentrations died before the end of yolk sac period

Table 2: Mean (±SD) comparison for *Clarias gariepinus* egg hatchability and larval survival at different nitrate concentrations

Nitrate concentrations (mg/L)	Hatchability (%)	Larval survival (%)
0.02	37.80^{x}±0.42	0^{x}
0.05	16.19^{m}±0.24	0^{x}
0.5	15.00^{n}±0.18	0^{x}
5	26.37^{z}±0.71	0^{x}
10	43.75^{e}±0.64	0^{x}
20	43.93^{e}±0.10	0^{x}
30	47.17^{d}±0.33	0^{x}
70	73.24^{a}±0.83	18.96^{b}±0.68
100	70.51^{b}±0.58	18.18^{c}±0.76
150	61.33^{c}±0.14	26.09^{a}±0.23
200	21.55^{r}±0.72	0^{x}
300	32.91^{y}±0.72	0^{x}
500	30.39^{y}±0.86	0^{x}
700	20.37^{s}±1.94	0^{x}

Mean values within each column which do not have the same superscript letter are significantly different ($p < 0.05$)

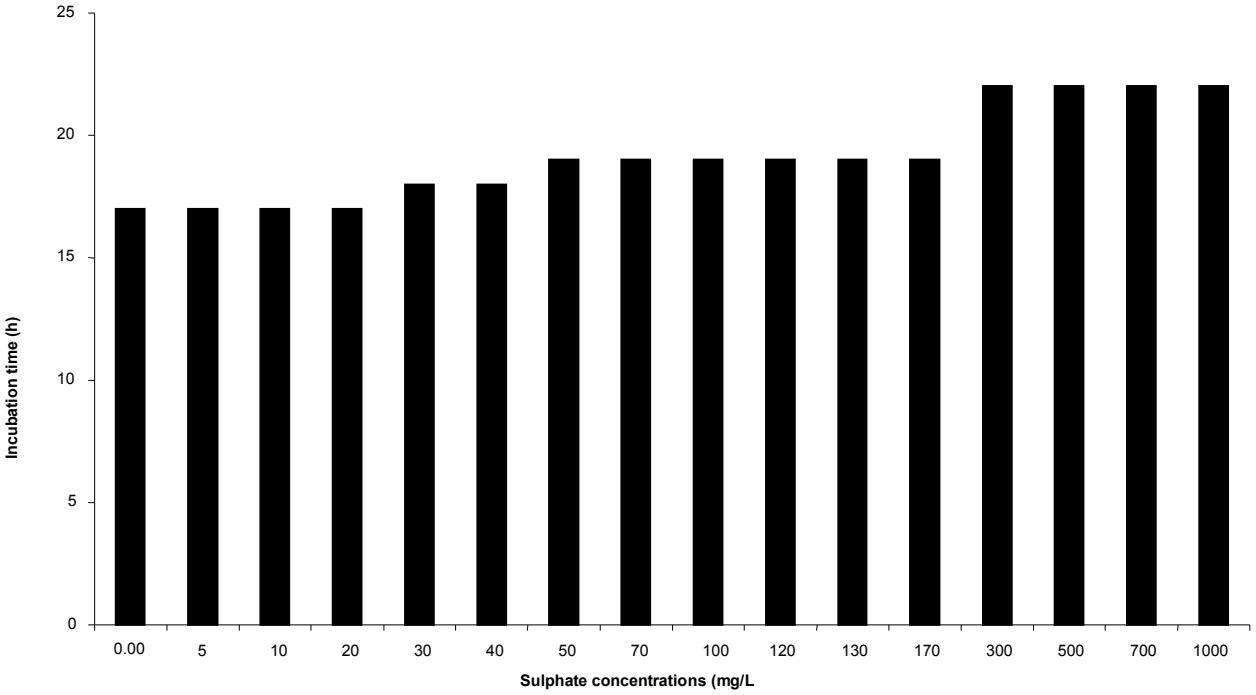

Figure 3: Incubation time to hatching of *Clarias gariepinus* eggs exposed to different sulphate concentrations

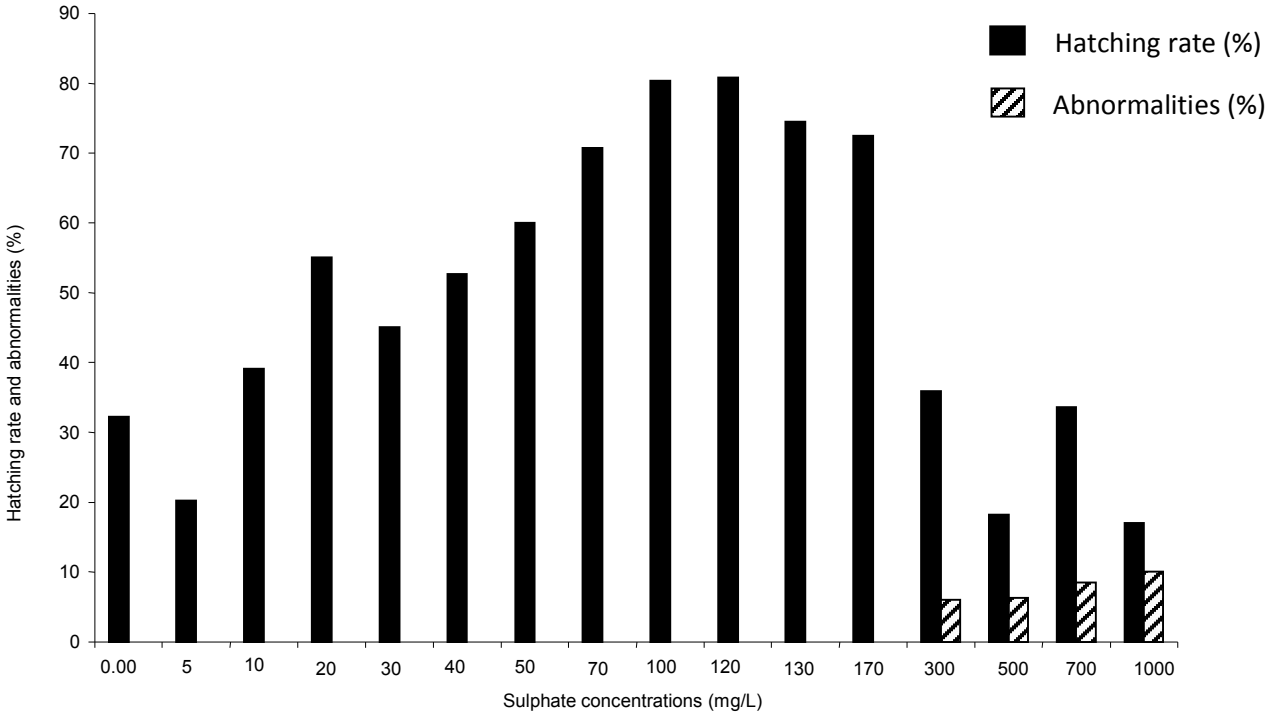

Figure 4: Hatching rate and percentage abnormalities of *Clarias gariepinus* eggs exposed to different sulphate concentrations

Table 3: Survival (mean ±SD) of *Clarias gariepinus* eggs and larvae exposed to different sulphate concentrations

Sulphate concentrations (mg/L)	Egg period			Hatching period		Yolk sac period		
	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 (%)
0.00*	124	40	32.26±0.98	35	87.50±1.21			
5*	163	33	20.25±1.39	16	48.48±0.91			
10*	92	36	39.13±1.23	23	63.89±0.50			
20*	100	55	55.00±0.06	53	96.36±0.21			
30*	82	37	45.12±1.25	36	97.30±0.61			
40*	93	49	52.69±0.74	45	91.84±0.57			
50	95	57	60.00±0.09	53	92.98±0.31	5	9.43±0.72	8.77±0.61
70	89	63	70.79±0.58	63	100.00±0.00	5	7.94±1.23	7.94±0.51
100	112	90	80.36±0.88	86	95.56±0.72	57	66.28±1.50	63.33±1.09
120	89	72	80.90±0.28	68	94.44±1.27	23	33.82±0.91	31.94±0.14
130	94	70	74.47±0.75	70	100.00±0.00	4	5.71±0.77	5.71±0.51
170*	98	71	72.45±0.78	71	100.00±0.00			
300*	92	33	35.87±0.20	25	75.76±0.42			
500*	88	16	18.18±2.89	13	81.25±0.47			
700*	140	47	33.57±0.76	41	87.23±0.38			
1000*	118	20	16.95±0.50	17	85.00±0.29			

*All yolk sac larvae at these concentrations died before the end of yolk sac period

Table 4: Mean (±SD) comparison for *Clarias gariepinus* egg hatchability and larval survival at different sulphate concentrations

Sulphate concentrations (mg/L)	Hatchability (%)	Larval survival (%)
0	32.26^{t}±0.98	0^{x}
5	20.25^{p}±1.39	0^{x}
10	39.13^{w}±1.23	0^{x}
20	55.00^{x}±0.06	0^{x}
30	45.12^{z}±1.25	0^{x}
40	52.69^{y}±0.74	0^{x}
50	60.00^{e}±0.09	8.77^{e}±0.61
70	70.79^{d}±0.58	7.94^{c}±0.51
100	80.36^{a}±0.88	63.33^{a}±1.09
120	80.90^{a}±0.28	31.94^{b}±0.14
130	74.47^{b}±0.75	5.71^{d}±0.51
170	72.45^{c}±0.78	0^{x}
300	35.87^{r}±0.20	0^{x}
500	18.18^{n}±2.89	0^{x}
700	33.57^{s}±0.76	0^{x}
1000	16.95^{m}±0.50	0^{x}

Mean values within each column which do not have the same superscript letter are significantly different ($p < 0.05$)

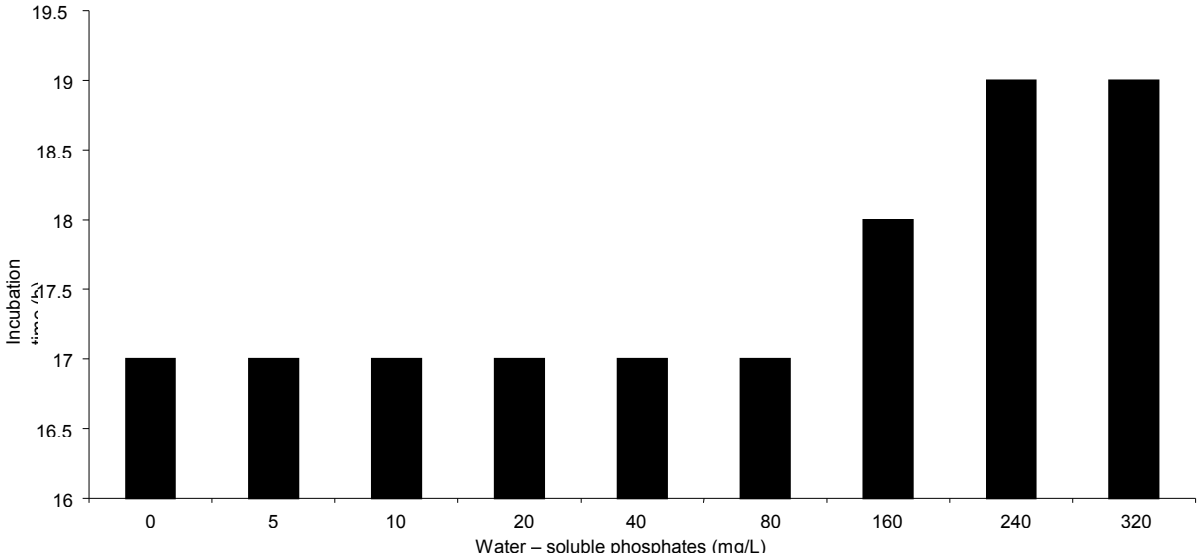

Figure 5: Incubation time to hatching of *Clarias gariepinus* eggs exposed to different levels of water soluble phosphate

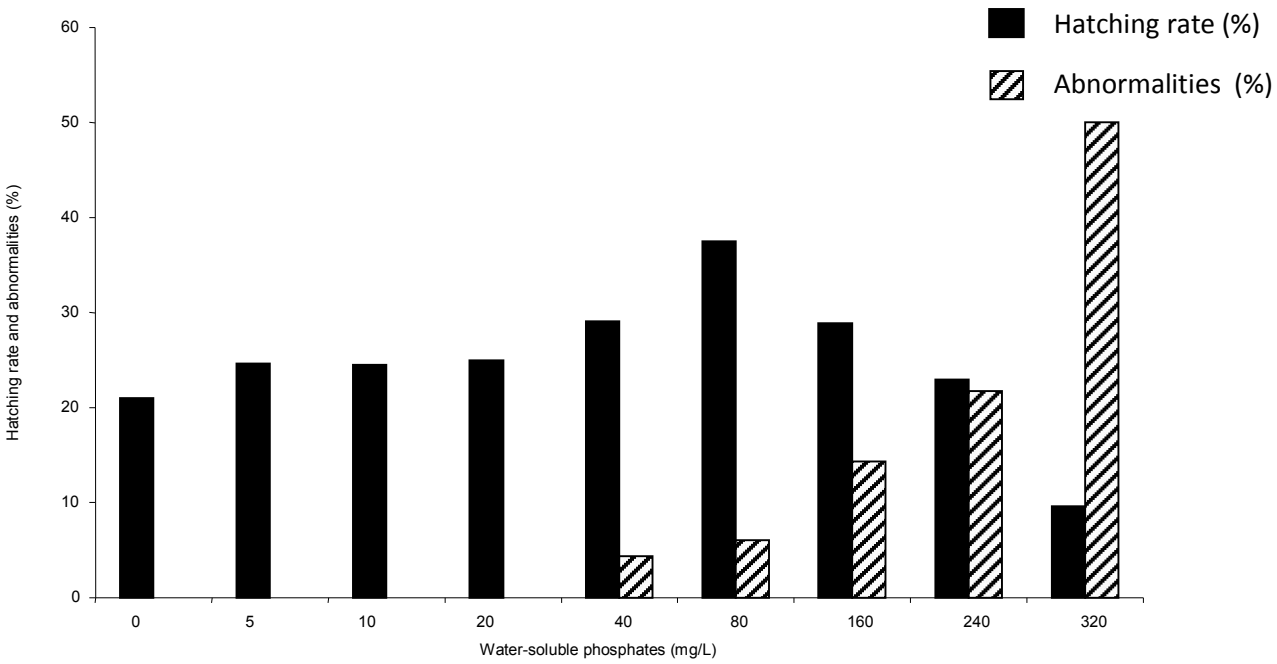

Figure 6: Hatching rate and percentage abnormalities of *Clarias gariepinus* eggs exposed to different levels of water-soluble phosphate

Table 5: Survival (mean ±SD) of *Clarias gariepinus* eggs and larvae exposed to different water- soluble phosphate

Water - soluble phosphate concentration (mg/L)	Egg period			Hatching period		Yolk sac period		
	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 (%)
0	81	17	20.99±1.06	13	76.47±1.02	4	30.76±0.61	23.55±0.64
5	65	16	24.62±0.54	13	81.25±1.10	5	38.46±0.91	31.25±0.81
10	102	25	24.51±0.14	20	80.00±0.20	10	50.00±0.13	40.00±0.37
20	92	23	25.00±0.10	23	100.00±0.00	12	52.17±0.24	52.17±0.47
40	79	23	29.11±1.29	17	73.91±0.70	3	17.64±0.36	13.04±0.18
80	88	33	37.50±0.92	29	87.88±0.17	3	10.34±0.78	9.09±0.20
160*[1]	97	28	28.87±0.23	21	75.00±0.28			
240*[1]	100	23	23.0±0.07	10	43.48±0.49			
320*[2]	104	10	9.62±0.54					
500	68	0	0					

No eggs hatched at 500mg/L

*[1] = All yolk sac larvae at these concentrations died before the end of yolk sac period

*[2] = All yolk sac larvae at this concentration died before the end of hatching period

Table 6: Mean (±SD) comparison for *Clarias gariepinus* egg hatchability and larval survival at different levels of water- soluble phosphate

Water-soluble phosphates (mg/L)	Hatchability (%)	Larval survival (%)
0	20.99^{e}±1.06	23.55^{d}±0.64
5	24.62^{c}±0.54	31.25^{c}±0.81
10	24.51^{c}±0.14	40.00^{b}±0.37
20	25.00^{c}±0.10	52.17^{a}±0.47
40	29.11^{b}±1.29	13.04^{e}±0.18
80	37.50^{a}±0.92	9.09^{x}±0.20
160	28.87^{b}±0.23	0^{y}
240	23.00^{d}±0.07	0^{y}
320	9.62^{x}±0.54	0^{y}

Mean values within each column which do not have the same superscript letter are significantly different ($p < 0.05$)

In conclusion, favourable and unfavourable nitrate, sulphate and phosphate levels for the embryonic development and survival of *Clarias gariepinus* were observed. The data suggest that the optimal normal hatching and larval survival of *Clarias gariepinus* may be expected if the concentration of nitrate is within the range of 70-150mg/L while that of sulphate is about 100mg/L and water-soluble phosphate is within 5-20mg/L during artificial propagation in the hatchery.

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