EFFECT OF SODIUM HYPOCHLORITE (NaOCl) ON FERTILITY, HATCHABILITY AND SURVIVAL OF FRY OF *CLARIAS GARIEPINUS*

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Abstract: The use of Sodium hypochlorite (NaOCl) often as disinfectants in hospitals in treating wastewater in order to prevent the spread of pathogenic microorganisms, causal agents of nosocomial infectious diseases and as bleaching agent for washing cloth have led to it been released in large quantity into the environment. This chemical finds its way into the aquatic ecosystem through runoffs.This aim of this study was to evaluate the effect of sodium hypochlorite (NaOCl) on fertility, hatchability and survival of *Clarias gariepinus* eggs exposed to sublethal concentrations of sodium hypochlorite after 28 days. The eggs of *Clarias gariepinus* were fertilized and incubated in sublethal concentrations (0.2 mg/L, 1 mg/L, 1.5 mg/L and 5 mg/L) of sodium hypochlorite and control. The percentage of fertilized eggs, percent hatchability and survival rate were calculated. There was concentration dependent reduction in hatchability of eggs and survival rates of fry exposed to sodium hypochlorite solution. These reductions were significantly difference for the different concentrations at $p < 0.05$. This implies that sodium hypochlorite inhibits hatchability and survival of *Clarias gariepinus* which could disrupt reproductive process of this species in the wild.

Keywords: *Clarias gariepinus*, Hatchability, Sodium hypochlorite, survival rate.

INTRODUCTION

Sodium hypochlorite (NaOCl, CAS No. 7681-52-9 and EC No. 017-011-00-1), a solution containing from 12.5% to 25% active chlorine gas (Cl_2), has a wide range of domestic, industrial, scientific and biomedical applications related to its biocide properties (Bron-deau *et al*., 2000). Approximately 70% of the total amount of sodium hypochlorite produced with bleaching, disinfecting and stain-removing properties is used to make bleach for household cleaners, laundry additives and for industrial processes. Household bleach usually contains approximately 5 - 10 % sodium hypochlorite while Industrial bleach contain up to 50 % sodium hypochlorite. Industrial processes such as commercial laundering, manufacturing of paper and pulp, for industrial chemical synthesis and disinfection of swimming pools and public water supplies are important applications of sodium hypochlorite.

The use of these chemicals in the environment is on the increase with its concomitant effects on aquatic lives. It is therefore, expected that Sodium hypochlorite will find its way into the aquatic environment when waste water containing it are released into the environment or from the disposal of their containers into the environment which is the common practice in developing country like Nigeria. When NaOCl is added to water and wastewater, the solution reacts readily with biological materials (including proteins and nucleotide bases) to produce a variety of organic chlorinated compounds (Emmanuel *et al*., 2004), which are mostly lipophilic, persistent, and toxic in aquatic environments (Debordea and Guntern, 2008).Sodium hypochlorite also produces chlorine gas when mixed with acidic products. Chlorine has high acute toxicity for aquatic organisms. Many toxicity values are less than or equal to 1 mg/l (Cárdenas *et al*., 2009). Papillomas of the oral cavity in fish have been associated with exposure to chlorinated water supplies (NTP, 1992, Emmanuel *et al*.,

2004). Low level chlorination (0.05 to 0.15 mg/l) results in significant shifts in species composition of marine phytoplankton communities (Emmanuel *et al*., 2004).

The increasing human and industrial use of sodium hypochlorite has led to the contamination of aquatic habitats which may have detrimental effects on fishes in Nigeria. The focus of this study is to determine the effect of Sodium hypochlorite (NaOCl) on fertility, hatchability and survival of *Clarias gariepinus* fry; which is directed in view of the economic importance and inherited scientific interest of the aquatic habitats to fisheries and water quality in order to ensure the sustenance of aquatic biota in Nigeria.

MATERIALS AND METHODS

Sources of Brood Stock organisms and Test materials. Male (1.8 kg) and female (1.3 kg) brood stocks were obtained from the pond in African Regional Aquaculture Centre (ARAC) Port Harcourt and taken to the hatchery. The hypo was procured from a local pharmaceutical shop in Port Harcourt.

Experimental Set Up. Fifteen tanks were set up to receive the fry; our concentrations of hypochlorite (hypo bleach) of 0.001 ml/L, 0.005 ml/L, 0.05 ml/L, 0.1 ml/L and control (0 ml/L) (control) with three replicates were used as treatment for the study.

The experiment was carried out between3rd Feb. 2016 and 3rd March, 2016. Syringe of 2ml and needle were used to obtain the volume of hypochlorite solution (hypo bleach) for different concentrations which was introduced into the water and made up to 5liters v/v.

Hormone Administration. The female was held on a working table with hand towel and was administered with a single dosage of 0.65ml of ovaprim to 1.3kg of weight to induce ovulation as recommended by the manufacturers. The hormone was drawn using 2ml of syringe and needle. Injection was carried out intramuscularly; a little distance from above the head beside the lateral line at an angle of 45^0C.The treated fish was then returned back into the container and covered with a perforated lid. The fish was injected at 9:40pm on 2nd Feb. 2015. The final maturation and ovulation of eggs was reached at 7:40am on 3rd Feb. 2015.

Collection of eggs and hatching. The female brood stock eggs were stripped and the eggs were collected with a dry small basin. The male was sacrificed to obtain the milt which was mixed in a basin containing saline solution (FAO, 1996).The eggs were introduced into the bowl by gentle stirring with plastic spoon; and ordinary water was added to effect fertilization. Immediately after fertilization, the eggs were scoop out with plastic spoon, counted and spread on a 2mm mesh net (a hatchery substrate called kakaban) in an incubation basin. The eggs were incubated in a basin containing measured hypochlorite made up to 5litre with distilled water. Hatching lasted for 48hrs and the newly hatched fry escaped through the 2mm mesh size net into the bowl underneath while the unhatched eggs remained on the net. Few hours after incubation, the observed white and opaque eggs were removed from each basin. This was done by siphoning out the dead/unfertilized eggs which appears whitish. The percentage fertilization was estimated as;

$$\text{fertilization\%} = \frac{\text{number of fertilized eggs}}{\text{total number of eggs incubated}} \times 100$$

Counting of Hatched Larvae. Twenty four hours after the incubation process, hatching began. The time interval for hatching of eggs varied with replicates. The hatched larvae were counted while the unhatched larvae were also counted and discarded; the percentage hatchability was estimated thus;

$$\text{Hatchability}(\%) = \frac{\text{no. of hatchedeggs}}{\text{total no. of fertilized eggs}} \times 100$$

unhatched eggs were removed from the incubator (basin) by siphoning. Feeding commenced 4 days after hatching at which time their yolk sac had been completely absorbed.

Fry were fed with small quantities of processed artemia, each replicate was feed 6times daily. Excess feed and waste were siphoned out using 5mm diameter hose.

The fry were monitored for twenty eight (28) days. Daily survivals were obtained through visual counting of the fry on a daily basis. The water was replaced every two weeks and the same quantity of hypochlorite solution (hypo bleach) as measured in the beginning of the experiment was added. The physicochemical parameters were monitored weekly. At the end of 28 days the fry(s) were counted and their percentage were estimated as;

$$\text{Survival}(\%) = \frac{\text{No. of fry at the end of study}}{\text{No. of fry at the begining of study}} \times 100$$

Physiochemical Parameters. The following physicochemical parameters were monitored in the hatchery: temperature, pH, nitrite nitrogen, Ammonia nitrogen, hardness, alkalinity, chloride using standard procedures as described by APHA, (1998).

Statistical Analysis of Data. Data were analyzed using one-way analysis of variance in completely randomized design. All analysis was done with the aid of Statistical Package for Social Sciences (SPSS) version 21.0.

RESULTS

Physico-chemical Parameters test solution

Table 1, shows the physicochemical parameters of water examined. The pH of the water was 7.0 which are neutral and fish can survive very well in it. Other physic-chemical parameters monitored during the experiment were within the tolerant and survival level of aquatic organisms.

Table 1.

Results of the Physico-chemical parameters of test solution

Water quality parameters	Values obtained
Temperature	$27.3^{oc} \pm 0.3$
pH	7.0 ± 0.4
Dissolve Oxygen (DO)(mg/l)	5.2 ± 0.7
Nitrite nitrogen(ppm)	0.05 ± 0.1
Ammonia nitrogen(ppm)	0.04 ± 0.4
Hardness(ppm)	5.0 ± 1.3
Alkalinity(ppm)	36 ± 3.3
Chloride(ppm)	28 ± 4.3

Hatchability of *Clarias gariepinus*. The result from this study revealed that hatchability decreases with increase in concentration of Sodium hypochloride solution. There was significant difference ($p < 0.05$) in the hatchability of the eggs from one concentration to the other.

Survival Rate of *Clarias gariepinus* after 28 days. The control (0.00ml/L concentration) had the highest survival rate of 69.70%, while at the highest concentration

(0.1) there was no survival at the end of the experiment. Survival rate continues to decrease as concentration increased which was statistically significant at $p< 0.05$ (Table 3).

Table 2.

Hatchibility of *Clarias gariepinus* eggs

Conc. NaOCl	Hatched	Unhatched	% Hatchability	Mean	SD
0	1500	1000	60.00	**1416.67**	**381.88**
	1750	750	70.00		
	1000	900	64.00		
0.001	500	2000	20.00	**510**	**25.46**
	540	1960	21.00		
	490	2010	19.60		
0.005	460	2040	18.40	**459.67**	**29.50**
	489	2011	19.54		
	430	2070	17.20		
0.05	370	2130	14.80	**372.67**	**16.16**
	358	2142	14.32		
	390	2110	15.60		
0.1	100	2400	5.00	**92.33**	**6.81**
	90	2410	3.60		
	87	2413	3.48		

Table 3.

Survival Rate of *Clarias gariepinus* after 28 days

Conc. Of NaOCL	Fry at the Beginning	No. That Survived	Mean/% Survival	Standard Deviation (SD)
0.00	1500	1000	987(69.70%)	**69.87**
	1350	912		
	1400	1050		
0.001	300	13	13(4.39%)	**1.53**
	289	12		
	301	15		
0.005	201	11	10(4.76%)	**1.00**
	219	9		
	211	10		
0.05	192	5	6(3.97%)	**1.53**
	153	7		
	108	8		
0.1	50	0	0(0.00%)	**0.00**
	47	0		
	32	0		

DISCUSSION

The temperature values of the test solution were within the range for a tropical climate in accordance with Federal ministry of environment (FMENV, 1999). An increase in temperature will lead to an increase rate of chemical reactions and formation of toxic complexes which may have profound effect on aquatic organisms. The pH of a water body could be affected by its age, geology and the chemicals discharged into it by communities and industries. The pH value for the water in this study was neutral which is also within the range that is tolerable of aquatic organisms and will not create problems for reproduction and survival (Ebong *et al*., 2006). The pH of an aquatic system is an indicator of the water

quality and the extent of pollution in the water shed (Jonnalagadda and Mhere, 2001). Dissolved oxygen (DO) concentration in natural waters depends on the physical, chemical and biochemical activities in the water body and DO of 5.2mg/l obtained in this study was within the permissible limit of the FMEV. Similar findings have been reported by some authors (Morenikeji and Raheem, 2008; Chukwu *et al.*, 2008 and Andem *et al.,* 2012). Other physico-chemical parameters monitored in this study are also with the tolerable limit of most aquatic organisms (WHO, 2003).

The hatchability of the eggs of *Clarias gariepinus* exposed to various concentrations of Sodium hypochlorite (NaOCl) shows that as the concentration increases the hatchability decreases and these differences were statistically significant. This work is also in agreement with Fasakin and Aberejo (2002) who accreted that pulverized powder of *Piper guineense* was effective and significantly inhibited egg hatchability and adult emergence of *D. maculatus* in smoked catfish. Also in agreement with this work is the work of Elmer-Rico and Micor (2007) on brine shrimp hatchability and lethality test assay used to determine the biological activity of the *Barringtonia asiatica* (Botong) seed's aqueous extract. They observed that at lower concentrations (1 – 50 ppm), there was a dose dependent relationship wherein the percentage hatchability decreased as the rate observed after the 12 treatment was reported to be probably due to an alteration in the development of Artemia (brine shrimp) embryos. Similar to *L. alopecuroides*, it is established also that, the water soluble fraction of crude oil contains active components including; xylene, naphthalene, benzene and toluene that can inhibit mitotic division in fertilized eggs of *Clarias gariepinus* resulting in non-hatchability due to abnormal development of the embryo. Mitotic inhibition of the fertilized eggs and abnormal differentiation and maturation could also result from the toxic effect of the harmful metallic ions like Vanadium, Nickel, Iron and Copper present in WSF of crude oil (Orlu and Ogbalu, 2013). The presence of sodium hypochlorite also affected the survival rates of the fry of *Clarias gariepinus* with the highest concentration recording zero survival. This has also been reported by other authors (Thurston *et al*., 1981, Elmer-Rico and Micor 2007). The result of this investigation is supported by the reports of Nwadukwe *et al*. (2006) on *Heterobranchus longifilis* and Onuoha and Nwadukwe (1990) on *Clarias gariepinus*.

CONCLUSION

The sublethal concentrations of Sodium hypochlorite can inhibit fertilization and hatchability of eggs and perhaps become toxic to *Clarias gariepinus larvae*, as well as, other non-target aquatic organisms. Waste water effluents containing this chemical should be treated before releasing it into the environment; in order to save the aquatic organisms from reduction in population.

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