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Influence of Salinity on the Developmental Stages of African Catfish (*Clarias gariepinus*)

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ABSTRACT: Salinity tolerance was tested in fertilized eggs, yolksac larvae, post yolksac larvae and fingerlings of *Clarias gariepinus* at 0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15ppt salinity. The highest hatchability was observed in fertilized eggs incubated in 2.5ppt, which recorded 71.8%. This was however not significantly higher than the 66.29% and 65.89% hatchability, recorded for the control group (Oppt) and 5ppt respectively. The median lethal salinity values in 96 hours (MLS-96) obtained for the yolksac larvae, post yolksac larvae and fingering stages are; 1.9, 2.2 and 7.8 respectively, demonstrated an ontogenetic variation in salinity tolerance of *C. gariepinus*. However, salinity tolerance of the fertilized eggs, larvae and fingerlings of *C. gariepinus* is observed to be generally low, and typically of fresh water stennohaline species. This relatively low salinity tolerance of *C. gariepinus* has probably prevented its existence in brackish waters. None of these stages are suitable for the transfer of *C. gariepinus* to brackish water environment for the purpose of commercial fish farming. However, results obtained in this study, could be a guide for the use of salt in the prophylactic and therapeutic control of pathogens sensitive to salt. The salinity tolerance of *C. gariepinus* is dependent on the developmental stage of the fish. © *JASEM, 1999.*

Clarias gariepinus is an economically important food fish found naturally in freshwater environment, and cultured primarily in freshwater ponds in tropical and subtropical countries of Africa and Asia. It's characteristic hardiness, resistance to disease, high yield potential and consumer acceptability (Salami and Fagbenro 1993), high fecundity and some degree of salinity tolerance has made it an aquaculture candidate.

Salinity tolerance of *C. gariepinus* has earlier been studied (Clay, 1977; Chervinski, 1984; Iyaji, 1986; Britz and Hecht 1989 and Oladosu, et al 1996), but the possibility of it's culture in the brackish water environment has not been fully explored. However, the extensive saline swamp of the Niger Delta, which have great potentials for aquaculture production have been estimated to be 730, 240ha (Pillay, 1973). These area have not been properly utilized due in part to the inadequate knowledge of many cultivable fish species suitable for this environment. Apart from the tilapias, no other group of brackish water fish species are hatchery bred in Nigeria hence, farmers depend on fingerlings from the wild to stock their ponds (Ezenwa and Ayinla, 1994). The technology for the artificial propagation of *C. gariepinus* has been well developed over the years, thereby ensuring ready availability of its seed.

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This study is therefore aimed at investigating the early developmental stage of *Clarias gariepinus*, most suitable for transfer to brackish water environment, for culture purposes.

MATERIAL AND METHODS

The studies were carried out at the catfish hatchery of the African Regional Aquaculture Centre, Aluu, Port Harcourt, Nigeria.

Broodstock Selection: Broodstocks of *Clarias gariepinus* used for the studies were selected from the stock maintained for commercial breeding by the Centre in its fresh water fish ponds.

Female broodfish were selected based on the observation of well rounded, soft and distended abdomen from which matured eggs were stripped by gentle application and of digital pressure according to Viveen, *et al* (1984). They were kept in separate 1,000L concrete tanks containing 15L of water. Male broodfish were selected based on the following criteria: possession of pointed and hyperaemic uro-genital palillae.

Induction of Ovulation: Four female broodfish weighing between 400-670g were injected with ovaprim[R], a synthetic analogue of gonadotropin releasing agent obtained from Syndel laboratories, Canada. dosage was calculated based on the manufacturer's recommendation of 0.5m/kg body weight of fish, and administered intramuscularly in the dorsal muscle mass as described by Viveen *et al* (1984).

Fertilization: Ovulated eggs were procured from the induced females by hand stripping. Males used were sacrificed and the testes dissected and sliced with razor along the edges, to extract milt into a 0.9% saline solution. Eggs from the females were pooled together and the extracted milt from the males was added to the egg mass and mixed gently but thoroughly. Fertilization was effected with addition of freshwater to the mixture of the eggs and the milt, and by gently mixing with a plastic spoon. this was continued for about two minutes after which the eggs were rinsed two times.

Incubation: Seven different salinity levels were prepared in triplicates, for all the tests on salinity tolerance of yolksac and post yolksac larvae. These include 0.0ppt, 2.5ppt, 5.0ppt, 7.5 ppt, 10.0ppt, 12.5.ppt and 15.0ppt. Known quantity of eggs were incubated in 2L of water of the different salinity values. Other water quality parameters including temperature, dissolved oxygen and pH were taken at incubation. Some fertilized eggs were incubated separately in freshwater for the salinity tolerance test in freshwater hatched yolk sac fry and fingerlings for the control.

Fingerling Production: The eggs incubated in freshwater-hatched 26 hours post incubation. The larvae absorbed their yolk by the third day and were nursed with egg yolk till day seven. An earthen pond ($50m^2$) which had been drained and limed to remove parasites and predators, 4 days earlier, was impounded and fertilized with pig manure and dry grasses. The 7 day old fry were stocked in the pond and nursed on ARAC fry diet (40% CP) for 28 days, to produce fingerlings.

Salinity Tolerance Test on Yolksac Larvae: Since the yolksac larvae emerged following hatching, the hatched larvae were left in the salinities incubation. Mortality was monitored daily for 4 days (96 hours), dead fish were counted and recorded on daily basis.

Salinity Tolerance Test on Post Yolksac Larvae: Twenty-five larvae each, that hatched out of eggs incubated separately in freshwater, were introduced in three replicates into water of 0.0, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 15.0ppt salinities, following the absorption of their yolks. They were fed *ad libitum* with egg yolk, and monitored for survival for 4 days. Daily mortality was recorded, and the water quality parameters including dissolved oxygen, pH and temperature were monitored.

Salinity Tolerance Test on Fingerling (28 days old): Fingerlings with average size and weight of 5.53cm (total length) and 1.33g respectively, were introduced into concrete tanks measuring 1m ×07m×0.6m and containing 500 litres of water of different salinities including 0.0, 2.5, 5.0, 7.5 10.0, 12.5 and 15.0ppt salinities, in two replicates. Before the commencement of salinity tolerance test, 5 randomly sampled individuals were weighed with a Lab top balance (Yamato LE 180) and measured for size using a meter rule. They were fed daily with ARAC growers mash (30% CP). Feeding commenced the day after they were introduced into the different salinity treatments. They were fed at 5% total biomass once daily. Dead fingerlings were counted, removed and recorded on daily basis. Temperature, dissolved oxygen and pH were monitored and recorded on daily basis.

Data collection and Analysis: The index for salinity tolerance used for the studies was the median lethal salinity -96 hours (MLS-96). It is defined as the salinity at which survival falls to 50%, 96 hours (4 days) following direct transfer from freshwater to various test salinities. It is regarded as the final survival (per cent) in each experimental salinity and calculated as the sum of the number of days each individual survived, divided by the product of total experimental days (4) and initial number of fish. Percentage survival was then plotted against the salinity of transfer. The MLS -96 was determined as the salinity at which survival fell to 50% (Watanabe, *et al* 1985).

The results of the salinity tolerance tests in different salinities and at different stages of development, were subjected to statistical analysis using One-way ANOVA.

Table 1: Percentage hatchability of *C. garlepinus* eggs incubated indifferent test salinities.

Salinity (ppt)	No. of eggs Incubated	No. of hatching produced	Hatchability* %
0	272.0	180.3	66.29^{ab}
2.5	247.3	177.7	71.86^{a}
5.0	202.3	133.3	65.89^{ab}
7.5	159.7	85.3	53.41^{b}
10.0	181.7	0	0^{c}
12.5	190.0	0	0^{c}
15.0	233.3	0	0^{c}

Values are means of three replicates.
*Means with the same prefix are not significantly different (P>0.05 and P.0.01)

RESULTS

Egg Hatchability: Hatching was observed to begin 23 hours and was completed 26 hours post incubation in all trials. The hatchability of eggs incubated in 2.5ppt salinity ranked highest with 72.86%, but was not significantly different from rates recorded for 0.0ppt and 5.0ppt treatments, where 66.29% and 65.89% were recorded respectively (Table 1). Seven and a half part per thousand (7.5ppt)

Table 2: Final survival of yolksac transferred from various test salinities to fresh water at the onset of hatching

Salinity	No. of eggs Incubated	No. of hatchings produced	Hatching	Percent Survival of yolk-sac larvae*			
				Day 1	Day 2	Day 3	Day 4
0	125.7	41.7	33.16\7	100^a	100^a	100^a	100^a
2.5	70.7	28.7	40.59	100^a	100^a	100^a	100^a
5.0	136	75.3	55.37	100^a	100^a	100^a	100^a
10.0	117	0	0	0^b	0^b	0^b	0^b
12.5	103	0	0	0^b	0^b	0^b	0^b
15.0	87	0	0	0^b	0^b	0^b	0^b

Values are means of three replicates
*Means in the same column with the same prefix are not significantly different (P>0.05 and P>0.01).

Table 3: Final survival of yolk-sac larvae transferred from various test salinities to freshwater at the completion of hatching

Salinity	No. of eggs Incubated	No. of hatchings produced	Hatching %	Percent Survival of yolk-sac larvae *			
				Day 1	Day 2	Day 3	Day 4
0	272	180.3	66.29	100^a	99.28^a	98.78^a	97.78^a
2.5	247.3	177.7	71.86	15.98^b	12.04^b	6.98^b	4.73^b
5.0	202.3	133.3	65.89	25.51^b	7.5^b	3.75^b	0^c
7.5	159.7	85.3	53.41	10.55^b	3.52^b	1.17^b	0^c
10.0	181.7	0	0	0c	0^c	0^c	0^c
12.5	190	0	0	0^c	0^c	0^c	0^c
15.0	233.3	0	0	0^c	0^c	0^c	0^c

Value are means of three replicates.
* Means in the same column with the same prefix are not significantly different (P>0.05 and P>0.01).

salinity recorded the least hatchability; 53.41%,, while no hatching was observed in 10.0ppt, 12.5ppt and 15.0ppt salinities. Hence, hatchability was significantly higher in 0.0ppt, 2.5ppt and 5.0ppt incubation media than in salinities of 7.5ppt to 15.0ppt (P>0.01 and P.0.05)00.

Fry Salinity Tolerance: The hatchlings transferred back to freshwater from various salinities at the onset of hatching were all to absorb their yolk, recording 100% survival up to day 4 post-hatch (post yolk sack stage) (Table 2).

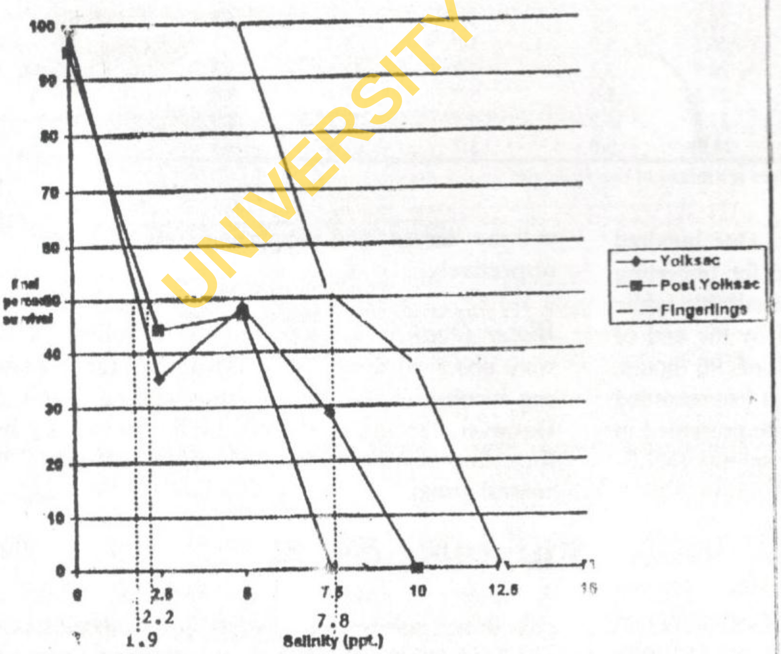

Figure 1: Mean lethal salinity for the different stages of development.

When hatchings are transferred back to fresh water from various test salinities at the completion of hatching, they retained their yolks and survival rates recorded were 97.78% for 0.0ppt, and 0% for 2.5 ppt, 5.0ppt and 7.5ppt by day 4 post hatch (Table 3). The same observations were made for yolksac larvae hatched and reared in the various test salinities. As shown in Table 4, with yolk retention, 100% mortality was recorded for salinities between 2.5ppt and 7.5ppt within four days. The calculated MLS-96 for the yolksac larvae was 1.9ppt as shown in Figure 1.

Table 4 shows the observations made during the salinity tolerance of post-yolksac larvae. Groups subjected to 10.0, 12.5 and 15.0pp salinities survived only for few hours, while those subjected to 2.5, 5.0 and 7.5ppt recorded 100% mortality within 4 days. Larvae reared in freshwater (0.0ppt) had 88% survival as at day 4 post-hatch. The calculated MLS-96 for the post yolk sac larvae was also 2.2ppt (Figure 1)

Table 4: Survival of yolksac larvae, post yolksac larvae and fingerling exposed to different test salinities for 96 hours (4 days).

Salinity (ppt)	Percent Survival											
	Yolksac larvae*				Post yolksac larvae*				Fingerlings**			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
0	100.0^{a}	99.28^{a}	97.78^{a}	97.78^{a}	100.0^{a}	92.0^{a}	88.0^{a}	88.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
2.5	38.54^{b}	2.2^{c}	0^{b}	0^{b}	54.6^{b}	17.2^{c}	5.2^{c}	0^{h}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
5.0	73.4^{ab}	20.0^{b}	0^{c}	0^{b}	48.0^{b}	28.0^{b}	12.0^{b}	0^{b}	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
7.5	9.1^{c}	5.6^{c}	0^{c}	0^{b}	0^{c}	0^{d}	0^{d}	0^{b}	70.0^{ab}	25.0^{b}	10.0^{b}	10.0^{b}
10.0	0^{d}	0^{d}	0^{c}	0^{h}	0^{c}	0^{d}	0^{h}	0^{b}	50.0^{h}	0^{c}	0^{c}	0^{c}
12.5	0^{d}	0^{d}	0^{c}	0^{h}	0^{c}	0^{d}	0^{d}	0^{b}	0^{c}	0^{c}	0^{c}	0^{c}
15.0	0^{d}	0^{d}	0^{c}	0^{b}	0^{c}	0^{d}	0^{d}	0^{b}	0^{c}	0^{c}	0^{c}	0^{c}

* Values are means of three replicates ** Values are means of two replicates
Means in the same column with the same superscripts are not significantly different (P>0.05 and P>0.01).

Table 5: Final percent survival and mean lethal salinity for the different stages of development

Salinity %°	Final Percent Survival		
	Yolk-sac Larvae Expt 1b	Post Yolk-sac Larvae	Fingerlings
0	99.26	95	100
2.5	35.28	44.31	100
5.0	48.35	47	100
7.5	28.65	0	51.25
10.0	0	0	37.5
12.5	0	0	0
15.0	0	0	0
MLS-96 ppt	1.9	2.2	7.8

Table 6: Water quality parameters during egg incubation, larvae rearing and fingerlings rearing in different test salinities.

Salinity (ppt)	Egg Incubation*			Larvae rearing*			Fingerlings**		
	Temp (°C)	Dissolved Oxygen (mgl^{-1})	Hydrogen ion conc.	Temp (°C)	Dissolved Oxygen (mgl^{-1})	Hydrogen ion conc.	Temp (°C)	Dissolved Oxygen (mgl^{-1})	Hydrogen ion conc.
0	26.7	5.9	6.1	26.2	5.3	59	27.1	5.9	6.9
2.5	26.7	6.1	6.3	26.4	5.7	5.7	27.0	5.6	6.8
5.0	26.8	5.7	6.4	26.5	5.7	5.7	27.7	5.7	7.0
7.5	26.8	5.8	6.1	26.9	5.7	5.7	27.6	5.8	7.6
10.0	27.0	5.3	6.2	27.5	5.9	5.7	27.4	8.0	7.0
12.5	27.2	5.5	6.1	27.8	6.2	5.7	27.3	8.3	6.9
15.0	27.4	5.7	6.2	28.0s	6.0	5.7	27.5	7.8	7.3

* Values are means of three replicates ** Values are means of two replicates

Salinity Tolerance of Fingerlings: One hundred percent (100%) survival was recorded for fingerling groups exposed to 0.0, 2.5 and 5.0ppt salinity, while 7.5 ppt group recorded 10% survival by the end of day 4. However, within the period of 96 hours, groups tested with 10.0, 12.5 and 15ppt had recorded 100% mortality. Details of the result are presented in Table 4. The calculated median lethal salinity (MLS-96) was 7.8ppt for fingerlings Figure1).

Ontogentic Variation in Salinity Tolerance: Table 5 summarises the calculated final percent survival and MLS-96 for the different development stages, exposed to different salinity. Salinity tolerance was observed to increase from yolksac larvae to post yolksac larvae, and from the latter to fingerlings. The MLS-96 values recorded (Figure 1) for yolksac is 1.9, while those observed for post yolksac larvae and fingerlings are 2.2 and 7.8 respectively.

Water Quality: Temperature and dissolved oxygen were observed to be within the normal limits during egg incubation fry and fingerling rearing (Table 6). However, the pH is slightly acidic for the fry and fingerling rearing in all treatments, including the control group.

DISCUSSION

A number of studies on the effect of salinity on growth and survival of a variety of fish species exist, and have been subjected to an extensive review by Brett (1979). Brett's analysis revealed that the highest growth rates of the various species considered clustered either around 0.0ppt (fresh water), 10 ± 2ppt, or 28-35ppt salinity. These clusters correspond roughly to three ecological groupings: *1. Freshwater*, *euryhaine species* and *stenohaline marine species*.

The observations made in the present study demonstrate substantially that *Clarias gariepinus* falls into the freshwater stenohaline group. Decreasing survival rates with increasing salinity as shown for *C. gariepinus* eggs, yolksac larva, post yolksac larva and fingerlings, appear to be characteristic of fresh water stenohaline fish, and it has been postulated that this is due to increasing maintenance requirements at higher salinities (Brett 1979 and Kilambi 1980).

The results obtained in this study agrees with earlier observations. A review of the literature shows that *C. gariepinus* larvae, juveniles and adults display a similar response to other freshwater stenohaline fish, in that they do not survive and grow for prolonged periods in salinities much above 10ppt.

The salinity tolerance of *C. gariepinus* does, however, appear to be dependant on the stage of development of the fish, since tolerance of the eggs, yolksac larvae, post yolksac larvae and fingerlings stages was observed to increase in that order, as evident in the MLS-96 values for the different stages of development. Similarly, an increase in salinity tolerance with stage of development had been documented for other freshwater stenohaline species, including channel catfish *Ictalurus punctatus* (Allen and Avault, 1969), buffalo fish; *Ictiobus cyprinellus* (Hollander and Avault, 1975, and roach; *Rutilus rutilus* (Schoefer, 1979).

Most information regarding the osmoregulatory physiology of teleost fishes is based on euryhaline fish, with the economically important salmonids having received most attention (Brett, 1979; Goswami, *et al* 1983; and Norton and Davis, 1977). Fish regulate their plasma ions such that the internal osmotic pressure of their fluids is equivalent to approximately 10ppt salinity, with a range of 2ppt depending on tolerance, regulating capacity, and environmental salinity (Brett 1979; Homes and Donaldson 1969). While a fairly large body of literature exists regarding osmotic and ionic regulation in fishes, explanations of the underlying mechanisms involved remain largely speculative (Evans 1980). It has been found that fresh water stenohaline fish are, in general, unable to maintain a constant concentration of body salts as the external salinity rises to concentrations isosmotic and hyperosmotic to their body fluid concentration (Goswami *et al*, 1983; Norton and Davis 1977; Maceina, *et al* 1980 and Ellory, *et al* 1972). Body fluids osmolarity increases as the salinity of the external medium is increased and the salinity tolerance of these fish is thus probably limited by the maximum osmotic pressure of the body fluid in which the cells can function (Maceina *et al.* 1980).

Holliday (1969) found that before spawning, gametes of teleosts are either isoosmostic with or hypoosmotic to the body fluid of the parent fish. The body fluid of *Clarias gariepinus* was observed to be hyperosmotic to fresh water, with the osmoconcentration of the blood plasma in fresh water being equivalent to 9.5ppt (Britz and Hecht, 1989). This explained the insignificant difference observed in the hatching rates of eggs of *Clarias gariepinus* artificially incubated in salinities of 0.0, 2.5, 5.0 and 7.5 ppt, and the significantly lower rates recorded for 10ppt (Oladosu *et al*, 1996). Egg mortality recorded in salinities higher than the osmotic concentration of the blood plasma of the parent fish was ascribed to dehydration, as evident in yolk retention, observed in the hatched fry by Oladosu *et al.* (1996).

The results of this study did not focus on growth rate and body fluid osmolarity, though, it could be said to be consistent with the interpretation that, survival rate of the tested stages of development of *Clarias gariepinus* was inversely related to salinity, and that salinity iso-osmotic with their body fluid concentration was lethal.

This relatively low salinity tolerance of *Clarias gariepinus* has probably prevented its penetration of more saline waters within its natural distribution, where more euryhaline species such as *Oreochromis mossambicus* exist.

The survival of *C. gariepinus* fingerlings in salinities up to 7.5ppt could facilitate the prevention and treatment of most freshwater ectoparastic pathogens. At the Rhodes University hatchery, infections due to Gyrodactylus, Dactylogyrus and Icthyophthirius have been successfully treated with salt (Bretz *el al*, 1989). Hence for prophylactic and therapeutic treatment of pathogenic infection, fertilized eggs, fry and fingerlings can be treated with low salinities (5ppt).

Based on the MLS-96 values obtained, salinity tolerance in *Clarias gariepinus* can be said to be influenced by stages of development. The optimal salinity ranges for egg incubation, fry nursing and fingerling rearing respectively, are 0-5ppt. Based on these observations, none of the developmental stages of *Clarias gariepinus* tested are suitable for transfer to brackish water environment for fish culture.

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