SALINITY TOLERANCE OF LARVAE OF AFRICAN CATFISH *Clarias gariepinus* (♀) X *Heterobranchus bidorsalis* (♂) HYBRID

[1]GABRIEL, Ugwemorubong Ujagwung, [2]GBULUBO, Awotongha Jacob and [1]DEEKAE, Suanu
[1]Department of Fisheries and Aquatic Environment, Rivers State University of Science and Technology, PMB 5080, Port Harcourt, Rivers State, Nigeria.
[2]African Regional Aquaculture Centre, Nigeria Institute of Oceanography and Marine Research, Aluu, Port Harcourt, Rivers State, Nigeria.

Corresponding Author: Gabriel, U. U. Department of Fisheries and Aquatic Environment, Faculty of Agriculture, Rivers State University of Science and Technology, PMB 5080, Port Harcourt, Nigeria. **Email:** ugwemg@yahoo.com **Phone:** +234 8032720882

ABSTRACT

Fourteen and twenty one day larvae were exposed to abrupt stepwise change in salinity (2, 4, 6, 8, 10 and 12 ppt) for 96 hours to determine mortality, median lethal mortality, MLS and median lethal time, MLT. The fourteen day-old fry that were exposed to 0 – 6 ppt recorded 90%, 87.5% 77.5% and 10% survival at the end of the 96 hours test period. Those that were subjected to 8 ppt and 10 ppt had 100% morality within 48 and 12 hours, respectively. The median lethal salinity at 96 hours (MLS-96) was 4.4 ppt which was about half the value for 6 ppt. The cumulative mortality of the graded salinities did not differ in 4 and 8 ppt but differed in 6 and 10 ppt, whereas the time intervals differed at 24 and 36 hours but did not differ at 48 – 96 hours, p<0.05. The 21 day-old fingerlings transferred into 8, 10 and 12ppt, recorded 100% mortality with 12, 3 and 2 hours, respectively. Those transferred into 0, 4 and 6 ppt had 100%, 80% and 10% survival, respectively at the end of 96hours test period. The median lethal salinity (MLS-96) for 6 and 12 hours were the same 7.19 ppt, while that for 48 hours was 4.79 ppt. The median lethal time (MLT_{50}) for 8 ppt was 8.55 hours, which was seven and half times the value for 4 ppt. The cumulative mortality the various salinity concentrations were not different in 10 and 12 ppt, but differ in 4 – 8 ppt, p<0.05. Mortality at various time intervals did not differ at 36 – 96 hours, but did at 3 and 6 hours, p<0.05.

Keywords: Catfish hybrid, Post larvae, Median lethal salinity, Median lethal time

INTRODUCTION

Clariid catfishes occur both in south-east Asia and in Africa and the highest generic diversity is on the African continent where some 14 genera have been reported (Teugels, 1984) against two in south-west Asia. In both continents, the clariids are of great economic important as food fish and for several years, they have been used in local fish culture where they proved to be fast growing protein source. They exhibit many qualities that make them suitable for culture. These include their hardy nature, disease resistance, tolerance to poor water quality, consumer acceptability, high fecundity, nutritional efficiency and attainment of large size within a short time (Hecht *et al.,* 1988; Haylor, 1989; Salami and Fagbenro, 1993).

Salinity is an important factor affecting the distribution of aquatic organisms. Depending on the concentration, it is known to affect the survival of the fish and other aquatic organisms by interacting with temperature, dissolved oxygen and other environmental factors (Eddy,

1981). The effects of physico-chemical parameters on cultured fish are of paramount importance and should be assessed in order to evaluate the optimum requirements and tolerances. The maximum and minimum levels of various environmental parameters such as: temperature, salinity, pH, dissolved oxygen (DO) concentrations, and ammonia among others are important in formulating culture practices of the organisms.

The osmotic pressure of water increases with increasing salinity (Boyd, 1982). Salinity therefore has been observed to influence growth, survival and production potentials of fish by its effect on osmoregulatory and metabolic activities of aquatic animals. At unfavourable concentrations, it can cause stress or even death of fish depending on the species (Eddy, 1981). Most estuarine and marine fish species which are euryhaline have optimal salinity range within which the scope for growth and other biological activities are optimal. Hence fish could be classified on the basis of their ability to tolerate environment differing in salinity. Thus, those that can tolerate wide range of salinity are termed euryhaline, while those with narrow range of salinity are stenohaline. Salinity tolerance tests with fish have been performed using different saline solution. Salinity tolerance tests with fish have been performed using different saline solutions for example, sodium chloride (Matern, 2001), diluted seawater (Chen and Chen, 2000) and synthetic seawater (Patridge and Jenkins, 2002). Study conducted indicated that mature catfish, *C. gariepinus* (0.6 – 1.5 kg) tolerated 10 ppt (25.6% seawater) for 100 hours with no sign of stress and with acclimatization the fish was able to tolerate 20ppt salinity (51.3%) seawater (Clay, 1977). Chervinski (1984) in his study of the salinity tolerance of young catfish (*C. gariepinus)* indicated that 95% of those on direct transfer tolerated 25% seawater (9.5%) but that no fish survived 30% seawater (11.7%), even though gradual increase. The medium lethal salinity (MLS_{50}) was 8 ppt using abrupt transfer and 10 ppt by gradual transfer (Iyagi, 1986). Britz and Hecht (1989) monitored the survival and growth of larvae of *C. gariepinus* between 0 and 5 ppt salinity.

The fingerlings of *H. bidorsalis* were found to resist changes from freshwater (0 ppt) up to 10 ppt (isosmotic) salinity without mortality (Fagbenro *et al.,* 1993). The present study was conducted to assess the tolerance of the post fry to salinity ranges which many occur in the natural habitat.

MATERIALS AND METHODS

Hybrid Procurement: Brood *C. gariepinus,* ♀ and *H. bidorsalis,* ♂ used for the study were obtained from a private fish farm at Aluu. The study was carried out in the hatchery and laboratory of African Regional Aquaculture Centre (ARAC) at Aluu, Port Harcourt from September to December, 2007. Gravid females of *C. gariepinus* (400 – 800 grams) were injected with ovaprim, a synthetic analogue of gonadotropin releasing agent. Dosage was calculated based on the manufacturer's recommendation of 0.5 ml/kg body weight of fish and administered intramuscularly in the dorsal muscle mass as described by Viveen *et al*. (1985). Ovulated eggs were stripped from the induced females into plastic bowl. Brood males of *H. bidorsalis* (0.6 – 1.3kg) were sacrificed and the testes dissected out. Incisions were made along the edges of the testes using a clean razor blade in order to extract the milt. The milt was squeezed out into a 0.9% saline solution. This was gently and thoroughly mixed. Fertilization was effected with addition of freshwater to the mixture of the eggs and milt, and by gently stirring with a plastic spoon.

The fertilized eggs were incubated in trays (30 x 45 cm^2) in a rectangular concrete tank containing freshwater. The hatched larvae were nursed in the concrete tank. The larvae were fed with *artemia* after the absorption of the yolk sacs up to the seventh day. The fry was then fed with ground and sieved Coppens feed (crude protein 45%) at 5% body weight for another week. Part of this was used for the salinity tolerance test for the fourteen day-old fry. The leftover in the concrete tank was fed with the same feed for another one week. These were used for the salinity tolerance test for the twenty one day-old fingerlings.

Saline Solution: Six salinity levels were prepared by weighting 2, 4, 6, 8, 10 and 12 g of sodium chloride with (Laptop balance Yamato LE 180, Yamato Scientific Company Limited, Tokyo, Japan) and dissolving each in a litre of freshwater to obtain 2, 4, 6, 8, 10 and 12 ppt, respectively. Freshwater was used as control (0ppt).

Salinity Tolerance test: Fingerlings each of fourteen day-old (1.22 ± 0.10 cm) and twenty one day-old (2.02 ± 0.2 cm) were introduced into each of the following test solutions 4, 6, 8, 10 and 12 ppt. The tolerance test was conducted in a five litre plastic aquarium containing three litre of the test solution. Each treatment level was replicated three times. The aquaria were covered with mosquito netting to prevent the fish from jumping out. Freshwater (0 ppt) was used as control. The fingerlings were not fed throughout the experimental period. Mortality was monitored at hourly interval for 96 hours. Dead fingerlings were removed, counted and recorded. Water temperature, dissolved oxygen and pH were determined twice a day. The test solution in each aquarium was changed daily. A fingerling was considered dead when opercular movement stopped and the fingerling did not react to gently prodding with a glass rod.

Water Quality: Mercury in glass thermometer was used to measure the water temperature during the salinity tolerance test for the fry and fingerlings of the African catfish hybrid. Hydrogen ion concentration (pH) was determined with handheld pH meter (Hannah Instruments Portugal, HA Model 191) which was calibrated against standard buffer solutions with pH values of 4, 7 and 10. Calibrations were carried done at each pH reading. Dissolved Oxygen was done with the Winkler method (APHA, 1985). Salinity was determined by a refractometer (ATAGOS/MILLE). The retractometer was first standardized with water from ARAC borehole to get reading of 0 ppt, before salinity of test solutions were determined.

Data Analysis: Probit analysis model (Finney, 1984) was used to determine the median lethal salinities (MLS) and median lethal times MLTs). Mortality at the various concentrations and duration were subjected to ANOVA and differences among means separated by Duncan multiple range test (Wahua, 1999). All analysis were done using Statistical Package for the Social Sciences, SPSS version 15 for Window.

RESULTS AND DISCUSSION

The water quality variables in the test aquaria were not different for the various salinity levels in the two life stages (Tables 1 and 2). The fourteen day-old fry that exposed to 0 – 6 ppt had 90%, 87.5% and 10% survival at the end of the 96 hour test period. Those subjected to 8 ppt and 10 ppt had 100% dead within 48 and 12 hours, respectively. The median lethal salinity at 96 hour (MLS_{50}) was 4.4 ppt which was about half the value for 6 hour (Table 3). However, the mortality at the exposure duration 48 – 96 hour was higher ($p<0.05$) from 3 – 36 hour for 14 day-old, whereas for 21 day-old mortality at 12 – 96 hour was similar but greater ($p<0.05$) than those at 3 – 6 hour (Table 3). The median lethal time (MLT_{50}) at 10 ppt was 11.65 hours which was about half the value for 6 ppt (Table 4). The percentage mortality of the 14 day-old larvae in this study declined with increased in salinity (Table 5). However, the survival at 48 – 96 hours were higher ($p<0.05$) compared to 3 – 36 hours (Table 5). For the 21 day-old, the 48 hour MLS_{50} was 4.79 (Table 6) and MLT_{50} at 8 ppt, 8.55 hour (Table 7). Percentage mortality increased with increase in the test salinity and at the exposure duration, and was similar at 12 – 96 hour and greater ($p<0.05$) than those at 3 – 6 hour (Table 8).

Salinity is one of the most important environmental factors exerting selective pressures on aquatic organisms and that organisms respond to varying salinity by either spending their life cycle in a single habitat where salinity is stable or variable; while others undergo ontogenic migrations with successive stages based on salinity regimes (Varsamos *et al.,* 2005).

The ability of each ontogenic stage to cope with salinity depends on the capacity to osmoregulate. Brett (1979) observed that observed that highest growth rates of various fish species relative to salinity clustered around 0 – 10 ± 2.00 or 28 – 35 ppt. These clusters correspond roughly to three ecological groupings: freshwater, stenohaline anadromous species; euryhaline and stenohaline marine species. The 21 day-old larva had higher survival when compared with the 14 day-old at 0 – 6 hour; however, at 8 and 10 ppt where 100% mortality was recorded in the 21 day-old it occurred at one-fourth and one-third respectively the time for that in the 14 day-old indicating the ontogenetic variation in salinity tolerance as reported in a number of fish species with increase in age (Murashige *et al*., 1991; Haddy and Pankhurst, 2004; Moustakas, *et al.,* 2004). However, the more rapid rate of death at 8 and 10 ppt in the 21 day-old is difficult to explain. The increased mortality ($p<0.05$) of the larvae in this study with increase in salinity was due to efflux of water out of the fish which has also been reported in larvae (Fashina-Bombata and Busari, 2003). The mortality at the exposure duration 48 – 96 hour was higher ($p<0.05$) from 3 – 36 hour for 14 day-old, whereas for 21 day-old mortality at 12 – 96 hour was similar but greater ($p<0.05$) than those at 3 – 6 hour and may suggest at that the larvae were able to attain internal homeostasis hence the rate of death did not differ as the exposure progressed with time.

Teleost embryos have developed strategies for coping with salinity challenge which includes formation of impermeable chorion and ion pump such as chloride cells, CC on the epithelium of embryos (Lin *et al.* 1999; Kaneko *et al.,* 2002). CC also played important roles in hydromineral and ionic homeostasis before tissues and organs for osmoregulation are developed (Ayson *et al.,* 1994*; Bone et al*., 1995). Besides, the 21 day-old may have developed tissues/organs in even miniature forms that have helped them cope better than the 14-day old. In some species such as *Mugil cephalus* (Lee and Menu, 1981), grouper-*Epinepalus coioides* (Yeh *et al.,* 1995) and black porgy- *Acanthopagrus schlegeli (*Chu *et al*., 1999) salinity tolerance seem to improve with age. The tolerance range of the larvae, 0 – 6 ppt with the optimum 2 – 4 and 2 – 6 ppt for 14 and 21 day-old, respectively indicated that the tolerance ranges were very narrow. The 96 hour MLS_{50} for the 21 day-old was 4.43 ppt; 48 hour MLS_{50} for 14 and 21 day-old were 4.52 and 4.79 ppt, a difference of 0.27 ppt suggesting the value was very narrow. Although the difference between the MLS_{50}, that between the MLT_{50} for 14 and 21 day-old larva (6.35 hour) at 8 ppt clearly showed that the older larvae was more tolerant. Decreasing tolerance with age is characteristic of freshwater stenohaline fishes possibly due to the physiological high energy cost of maintaining internal hydromineral and ionic balance in the face of increasing salinity (Kilambi, 1980). Hence several studies have confirmed that the early life stages of *Clarias and Heterobranchus* sp. hardly survive and grow for a long period beyond salinity above 10 ppt (Iyaji, 1986; Fagbenro *et al.,* 1993*).* Under salinity challenge it appears the body fluid osmolality of the larvae are raised relative to that of the external medium. Thus their tolerance is probably limited by the maximum osmotic pressure of the body fluid in which the cells can function (Maceina *et al*., 1980).

The fingerlings of the parent stocks, *H. bidorsalis* and *C. gariepinus* had been classified as freshwater stenohaline fishes (Fagbenro *et al.,* 1993; Oladosu *et al.,* 1999) which is true of larvae from their hybrid. A study by Odiete and Jacob (1985) indicated that the skin of the parents stock, *Clarias* sp. was non-keratinized with 25 – 32 layers of cells which make it permeable to Na^+ and Cl^- compared with that of another catfish, *Chrysichthys* sp. having keratinized layers that are impermeable to water and electrolytes. Hence, the fish tolerates wide salinity ranges even as was evidenced in fingerling *C. nigrodigitatus* that tolerated up to 22 ppt salinity (Anyanwu*,* 1991) unlike the clariids. The nature and functions of the integument of the larvae in this study may be similar to that of the parents and hence their response.

Laboratory studies on the effects of changing salinity on the early life stages of a number of freshwater fish species are meant to

Table 1: Water quality variables of the various test salinities for 14 day old *C. gariepinus* larvae

Salinity (‰)	Temperature (°C)		Dissolved oxygen (mg/l)		pH	
	Range	Mean	Range	Mean	Range	Mean
0	25.5-27.5	26.6±0.74	5.2-6.2	5.8±0.37	5.6-6.2	5.8±0.26
2	26.0-27.5	26.7±0.47	5.8-6.2	5.9±0.16	5.8-6.2	6.0±0.14
4	25.5-27.5	26.7±0.69	6.0-6.2	6.1±0.10	5.8-6.4	6.2±0.21
6	26.5-27.5	27.0±0.40	6.2-6.4	6.3±0.10	5.8-6.4	6.1±0.19
8	26.5-27.5	27.2±0.38	5.8-6.4	6.1±0.22	5.8-6.4	6.2±0.23
10	26.0-27.0	26.5±0.34	6.0-6.4	6.1±0.16	5.8-6.2	5.1±0.16

Table 2: Water quality parameters of the various test salinities for 21 day old *C. gariepinus* larva

Salinity (‰)	Temperature (°C)		Dissolved oxygen (mg/l)		pH	
	Range	Mean	Range	Mean	Range	Mean
0	26.0-27.0	26.5±0.46	6.0-6.2	6.1±0.10	5.6-6.2	5.9±0.22
4	25.5-27.0	26.1±0.68	5.8-6.2	6.1±0.17	5.8-6.2	6.1±0.17
6	25.5-27.0	26.5±0.76	5.8-6.2	5.9±0.16	5.6-5.8	5.7±0.10
8	26.5-27.0	26.2±0.34	5.6-6.2	5.8±0.21	5.4-6.2	5.8±0.29
10	26.5-27.0	26.5±0.44	6.2-6.8	6.4±0.24	6.2-6.8	6.4±0.0.24
12	25.0-27.0	25.8 ±0.68	6.2-6.6	6.4 ±0.14	6.2-6.6	6.4 ±0.16

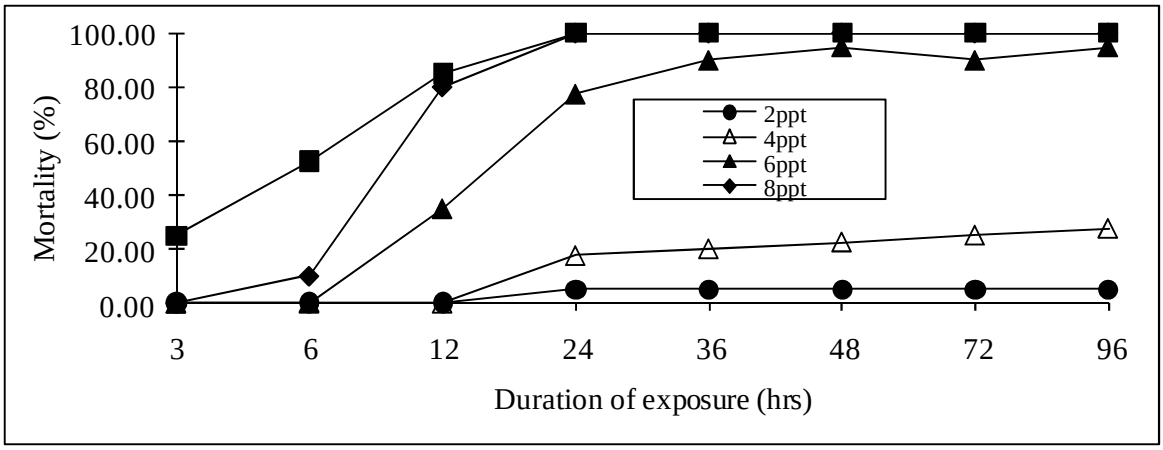

Figure 1: Mortality (%) of 14-day old larvae of *Clarias gariepinus* exposed graded levels of salinity under laboratory conditions

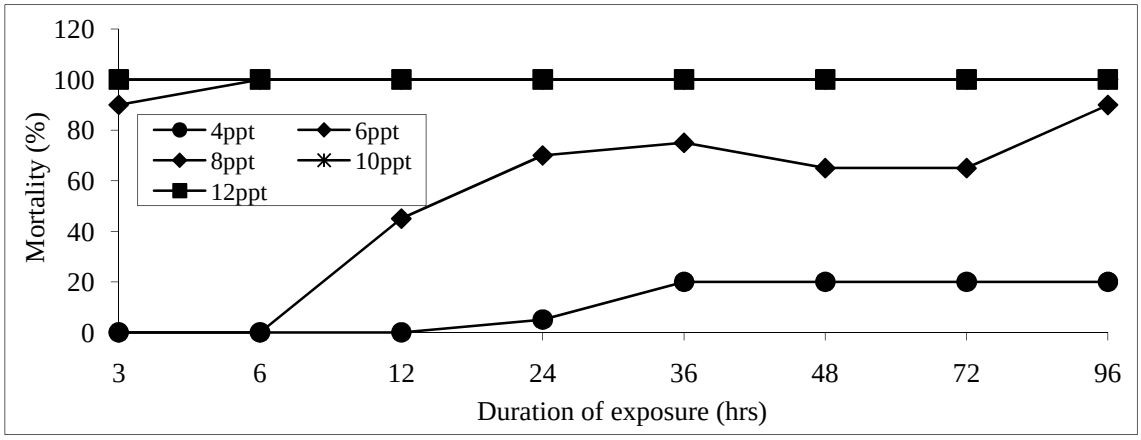

Figure 2: Mortality (%) of 21-day old larvae of *Clarias gariepinus* exposed graded levels of salinity under laboratory conditions

Table 3: Median Lethal salinity, MLS and associated 95% lower and upper confidence bounds (limits) of 14 day- old hybrid fry (*C. gariepinus* ♂ x *H. bidorsalis* ♀)

Duration (hours)	MLS_5	MLS_{50}	MLS_{85}	MLS_{90}	MLS_{95}	MLS_{99}
6	7.85 (6.68-8.41)	9.80 (9.41-10.34)	11.02 (10.45-1125)	11.31 (10.67-12.72)	11.74 (10.99-13.43)	12.55 (11.58-14.77)
12	5.19 (4.30-5.75)	7.38 (6.99-7.77)	8.75 (8.30-9.43)	9.08 (8.58-9.85)	9.56 (8.98-10.49)	10.47 (9.72-72-11.70
24	2.96	5.36	6.87	7.23	7.76	8.76
48	2.06 (-2.02-3.30)	4.52 (241.6 (3.28-5.93	6.07 (4.97-9.23)	6.44 (5.26-10.12)	6.98 (5.67-11.47)	8.00 (6.38-14.04
72	2.00 (-1.42-3.17)	4.48 (3.36-5.71)	6.03 (5.00-8.68)	6.40 (5.30-9.12)	6.98 (5.67-11.47)	7.97 (6.44-12.98)
96	1.94 (-095-3.05)	4.43 (143-5.52)	6.00 (5.04-8.23)	6.37 (5.34-8.95)	6.92 (5J6-10.05)	7.95 (6.5042.14)

Table 4: Median lethal time, MLT and associated 95% lower and upper confidence bounds (limits) of 14 day- old hybrid fry (*C. gariepinus* ♂ x *H. bidorsalis* ♀)

Duration (hours)	MLT_5	MLT_{50}	MLT_{85}	MLT_{90}	MLT_{95}	MLT_{99}
2	38.7 (13.55-58.96)	172.99 (124.97-394.41)	257.82 (177.34-640.69)	277.88 (189.59-699.06)	307.61 (207.71-785.64)	363.39 (241. 60-948.12)
4	24.60 (12.30-41.53)	132.77 (106.97-201.84)	200.93 (153.81-329.80)	217.05 (164.92-360.23)	240.94 (181.32-405.40)	285. 76 (211.99-490.22)
6	21.4	29.99	62.47	70.15	81.53	102.89
8	12.02 (9.34-13.89)	17.48 (15.77-19.37)	20.92 (19.07-23.57)	21.74 (19.79-24.63)	22.94 (20.84-26.21)	25.20 (22.73-29.25)
10	8.74	15.77	11.65	13.49	13.92	14.57

Table 5: Cumulative mortality associated 95% upper and lower confidence bounds of 14d-old larvae of catfish hybrid exposed to 9a) graded salinity at (b) various durations

(a) Salinity (‰)	Cumulative mortality	95%confidence bounds		(b) Duration	Cumulative mortality	95%confidence bounds	
		Upper	Lower			Upper	Lower
4	2.97[c]	1.98	3.96	3	1.20[d]	-0.10	2.50
6	1.47[b]	0.47	2.47	6	1.26[d]	0.02	2.48
8	3.50[c]	2.52	4.49	12	2.60[c]	1.30	3.90
10	8.03[a]	7.04	9.03	24	5.60[e]	4.34	6.84
4	2.97[c]	1.98	3.96	36	4.05[b]	2.79	5.31
6	1.47[b]	0.47	2.47	48	6.00[a]	4.74	7.26
8	3.50[c]	2.52	4.49	72	6.45[a]	5.19	7.71
10	8.03[a]	7.04	9.03	96	6.50[a]	5.24	7.76

Means with similar alphabets in the same are not significantly different 9p>0.05) for salinity and duration respectively.

Table 6: Median Lethal salinity, MLS and associated 95% lower and upper confidence bounds (limits) of 21 d- old hybrid fry (*C. gariepinus* ♂ x *H. bidorsalis* ♀)

Duration (h)	MLS_5	MLS_{50}	MLS_{85}	MLS_{90}	MLS_{95}	MLS_{99}
6	5.72 (4.91-6.18)	7.18 (6.83-7.51)	8.09 (7.73-8.64)	8.31 (8.19-8.94)	8.6 (8.19-9.38)	9.24 (8.68-10.24)
12	4.74 (3.80-5.35)	7.19 (6.78-7.60)	8.73 (8.24-9.4)	9.09 (6.55-9.91)	9.63 (9.00-0.61)	10.64 (9.84-11.96)
24	4.53 (3.35-5.00)	5.66 (5.29-5.93)	6.38 (5.09-6.905)	6.55 (6.23-7.24)	6.80 (6.42-7.68)	7.27 (6.77-8.59)
48	2.25 (2.42-3.72)	4.79 (4.45-5.12)	5.79 (5.40-6.34)	5.99 (5.59-6.65)	6.33 (5.80-7.13)	6.97 (6.39-8.04)
72*						
96 *						

**Percentage responding in all salinities is the same.*

Table 7: Median Lethal Time, MLT and associated 95% lower and upper confidence bounds (limits) of 21 day-old hybrid fry (*C. gariepinus* ♂ x *H. bidorsalis* ♀)

Salinity (‰)	MLT_5	MLT_{50}	MLT_{85}	MLT_{90}	MLT_{95}	MLT_{99}
4	19.05 (9.15 -26.34)	65.06 (59.57 -71.53)	96.06 (85.72-105.62)	100.92 (91.67-133.92)	111.08 (100.41-126.29)	130 (116.67-149.60)
6	29.79 (18.97-52.57)	34.10 (24.05 -87.83)	74.36 (47.11-423.31)	83.88 (53.72-512.87)	98.00 (62.09-164.46)	124.47 (78.21-898.21)
8	-11.13	8.55	18.6	21.40	25.45	33.01
10*						
12*						

**Percentage responding in all salinities is the same.*

Table 8: Cumulative mortality associated 95% upper and lower confidence bounds of 14 day old larvae of catfish hybrid exposed to 9a) graded salinity at (b) various durations

(c) Salinity (‰)	Cumulative mortality	95% confidence bounds Upper	Lower	(d) Duration	Cumulative mortality	95%confidence bounds Upper	Lower
4	1.00d	0.75	1.25	3	3.67e	3.38	3.95
6	5.56c	5.32	5.81	6	4.83d	4.55	5.12
8	8.88b	8.63	9.12	12	5.50b	5.21	5.79
10	10.00a	9.75	10.25	24	6.25	5.96	6.54
12	10.00a	9.75	10.25	36	6.50	6.21	6.79
4	1.00d	0.75	1.25	48	6.83a	6.55	7.12
6	5.56c	5.32	5.81	72	6.83a	6.55	7.12
8	8.88b	8.63	9.12	96	6.83a	6.55	7.12

Means with similar alphabets in the same are not significantly different 9p>0.05) for salinity and duration respectively

assess the possibility of introducing such species into non-natural ecosystems especially with the salinization of rivers and wetlands which is a major environmental concern in many parts of the world (Williams, 1987). A most common approach to laboratory measure of salinity tolerance is the concentration that is lethal to 50% of the individuals (the LC_{50}) over a period of time usually 48 – 98 hours. Kefford *et al.* (2004) noted that the values are usually determined from three broad methods: direct transfer (direct LC_{50}), slow acclimation (slow LC_{50}) and early life stage or early LC_{50} which gives very large differences in the LC_{50}. Besides, the studies are conducted without other stressors, quite contrary to what obtain in natural ecosystems. The authors observed that "survival in laboratory experiment may not predict survival in nature, let alone the maximum salinity that could support a self sustaining population" Besides, although laboratory measurements of sublethal effects are possible, it is difficult to relate to the salinity at which a species can maintain a self sustaining population in nature (Brinkhurst *et al.,* 1983). Hence, although the larvae in this study had optimum salinity tolerance range at 0 – 4 ppt, in the natural environment with the vagaries in physico-chemical and other environmental factors the optimum range may be greatly influenced. The farmer intending to introduce the larvae to the optimum salinity in the natural environment should carry out a field validation study as suggested by Connell *et al.* (1999) so as avoid heavy losses that may discourage such attempts and the associated benefits.

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