JFLS | 2020 | Vol 5(2) | Pp 1-9

Research Article

Salinity tolerance of Stinging catfish, *Heteropneustes fossilis* at different ontogenetic stages

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ISSN: 2456-6268

ARTICLE INFO
Received: 08 July 2020
Accepted: 06 October 2020
Available online: 01 December 2020

KEYWORDS
Heteropneustes fossilis
Salinity tolerance
Ontogenetic stages
LC_{50}

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ABSTRACT

The present work intended to evaluate the salinity tolerance of the freshwater fish *Heteropneustes fossilis* at different ontogenetic stages, seeking alternative approaches to freshwater aquaculture in a challenging climate-change scenario for sustaining aquaculture production through static bioassay performance. *H. fossilis* of three different growth stages of 5-day larvae, 21-day fry, and 45-day fingerling were exposed to different salinity concentrations of 0 (T_1), 2 (T_2), 4 (T_3), 6 (T_4), 8 (T_5), 10 (T_6) and 12 (T_7) ppt for 96 h to determine the median lethal concentration (LC_{50}). No mortality was found in control group (0 ppt) at all the three stages during the exposure. In case of larvae, 100% cumulative mortality was found among 6 (T_4), 8 (T_5), 10 (T_6) and 12 (T_7) ppt within 84 h, 36 h, 3 h and 0.5 h of exposure, respectively while fry showed 100% mortality at 12 ppt (T_7) within 36 h of exposure. The LC_{50} values for 24 h, 48 h, 72 h and 96 h exposure times were 6.61 ppt, 4.07 ppt, 2.95 ppt and 2.82 ppt, respectively for larvae; 10.71 ppt, 9.54 ppt, 8.51 ppt and 7.58 ppt, respectively for fry; and 22.38 ppt, 12.58 ppt, 11.48 ppt and 10 ppt, respectively for fingerling stages. The LC_{50} value after 96 h revealed higher tolerance among 45-day old fingerling (10 ppt) than 21-day old fry (7.58 ppt) and 5-day old larvae (2.81 ppt), demonstrating that survival in saline water depends on their age at initial exposure to low salinities. Normal feeding and behavioral response of fish were observed from 2 to 6 ppt salinity. The result suggests, *H. fossilis* is a potential candidate for aquaculture in slightly brackish water areas (2-6 ppt) of Bangladesh.

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INTRODUCTION

Bangladesh's flat, low-lying deltaic land and geographical location makes her highly vulnerable to a combination of climatic variables, including cyclone, drought, flood, rainfall, salinity and sea level rise. According to the Global Climate Risk Index, Bangladesh is ranked sixth among countries vulnerable to climate change, while it was ranked first in 2012 (Harmeling and Eckstein, 2012).

Salinity intrusion is one of the utmost key parameter which is changing primarily for climate change. Saltwater from the Bay of Bengal has reached over 100 km inland (DMB, 2010). Cyclones with tidal surges, sea level rise, tidal flooding during wet season and withdrawal of freshwater inflows from upstream are likely to play a critical role in salinity intrusion. The problem becomes exacerbate especially during the dry season when rainfall is inadequate and incapable of lowering the concentration of salinity on surface water and leaching out salt from soil. Reduction of freshwater availability by salinity intrusion and its associated hazards in the coastal area are increasing with the increasing of sea level rise. Cyclone, Sidr (15 November, 2007) and Aila (27 May, 2009) hit South and South Western part of Bangladesh and destroyed the coastal embankment infrastructure and increased the salinity. Salinity has recently been increased in coastal rivers to 4 ppt in the monsoon and 13 ppt in the dry season (Khan *et al*., 2011). About 1.06 million ha of land in coastal Bangladesh have been affected by salinity (FPMU, 2013) which is predicted to increase two million ha by 2050 (Conway and Wage, 2010).

Even though salinity intrusion is a slow process but the impact is devastating. Increasing salinity level due to sea level rise will affects the fish production directly or indirectly by altering the ecosystem through change in water quality can instigate a change in species composition and distribution especially in coastal areas because of the loss of nursery ground and loss of shelter. There will be a clear change in seasonal abundance of individual fish generating

changes in the ecological factors. Salinity intrusion will affect the physiology and sex ratios of fishes, alter timing of spawning, readiness, maturity and gonad development of fishes in breeding season. A change in salinity effects the survival and growth of fish (Muir and Roberts, 1995). Due to these high levels of salinity, the traditionally cultured fish species fall under stress and could not perform well resulting stunted growth and low production. Since salinity tolerance of fish is often positively correlated to growth capacity (Lemarie *et al.*, 2004) and metabolism (Sun *et al.*, 2006). Higher water salinity level may bring changes in migrations, and/or peak abundance, increase invasive species, diseases and algal blooms. These will lead to changes in timing and levels of productivity across marine and freshwater systems and reduced production of target species in marine and freshwater systems. Thus strictly freshwater Cyprinids, Anabantids, Channidae and many other fishes may likely suffer most (WFC, 2007).

The shortage of freshwater in many countries, together with the competition requirements for agriculture and other urban activities has increased the pressure to develop aquaculture in brackishwater and seawater although the effect of salinity on aquatic organisms are still undervalued. Most of the country takes initiative to adopt this adverse condition by doing several research works on salinity and its effect on aquatic organism but unfortunately Bangladesh is far away from this track. So immediate research should be needed to resolve the production loss and as well as extinction of fish species due to salinity increase. For the aquaculturists, it is important to determine the optimum level of salinity for the cultured fishes.

In Bangladesh coastal aquaculture mainly includes shrimp and prawn culture but other fish species have not been cultured till now. If the salinity tolerance limit of different commercially cultured freshwater fish species is known they can be easily cultured in brackishwater aquaculture system and the adaptive strategy of freshwater fish species can be said. Salinity tolerance limit of few freshwater species have been identified (Dubey *et al.*, 2015; Dubey *et al.*, 2014; Islam *et al.*, 2014; Sarma *et al.*, 2013). But the salinity tolerance limit of different life stages of a fish species is not conducted yet. If we know the salinity tolerance limit of different life stages of a fish species, we can grow it in freshwater at certain level and then transfer it to brackish water for culture purpose.

The stinging catfish *H. fossilis*, localy known as "shing fish", is an indigenous air-breathing species and commercially important for its good taste and nutritional point of view (Ali *et al.*, 2018 and Kumar *et al.*, 2017). Shing is rich in protein and have very high content of iron (226mg/l0gm) and fairly high content of calcium compared to many other freshwater fishes. Due to its special nutritive value and medicinal quality it is good for patient. Being a lean fish (fat content only 2.57% ± 0.24 on fresh weight basis) it is very suitable for those people whom are unable to utilize animal fats (Rahman *et al.*, 1982). This is primarily a fish of ditches, beels, swamps, ponds and marshes, but sometimes found in muddy rivers (Froese and Pauly, 2012). Commonly, during the dry season *H. fossilis* lives in semiliquid and semi-dry mud, and even when the mud dries up they take their bodies to the bottom of fissures and crevices formed by the cracking mud. It is also able to tolerate slightly brackishwater (Ahmmed *et al.*, 2017). The present experiment was conducted to determine the salinity tolerance level of *H. fossilis* at different ontogenetic stages (larvae, fry and fingerling) at different salinity exposure.

MATERIALS AND METHODS

The experiment was carried out at the laboratory of the Department of Fisheries Biology and Aquatic Environment, Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. *H. fossilis* 5 days larvae (0.77±0.21 cm; 0.02±0.06 g), 21 days fry (3.04±0.65 cm; 0.28±0.06 g) and 45 days fingerling (8.20±0.58 cm; 2.41±0.31 g) were collected from Authentic Hatchery and Sharnalata Agro-Fisheries Ltd. located in Mymensingh district. After transportation in oxygenated plastic bags to BSMRAU campus, the fish were acclimatized for a period of 12 h prior to start of each experiment.

Preparation of test solution

In this study, concentrated brine (125 ppt.) was obtained from salt-bed of Cox's Bazar. To obtain the required salinities, different proportions of the brine water was mixed with tap water. All salinity gradients were adjusted gradually by adding brine water with tap water. Salinity was determined by using portable refractometer (Optika, HRD, Italy).

Experimental Design and Procedure

For each of three ontogenetic stages (larvae, fry and fingerling with a stocking density 1.5/L) of *H. fossilis*, seven different salinity levels of 0 ppt (T_1), 2 ppt (T_2), 4 ppt (T_3), 6 ppt (T_4), 8 ppt (T_5), 10 ppt (T_6) and 12 ppt (T_7) with four replicates were assigned to Completely Randomized Design (CRD).

The acute salinity test on *H. fossilis* was conducted following the OECD Directive No. 203 (1992) 'Fish, acute toxicity test'. A static acute integrated bioassay was performed to determine Median lethal concentration (LC_{50}) of salinity. 15 numbers of uniform sized pre-acclimatized *H. fossilis* were directly exposed to the different salinity levels (0, 2, 4, 6, 8, 10 and 12 ppt) for 96 h for determination of LC_{50}. Mortalities were recorded, dead fish if any, were removed immediately. Fish were considered as dead when respiratory movement of the opercula stopped and there was no response to touch. Accumulated mortality data were analyzed following probit analysis method (Finney, 1971). Aeration was applied to the container in order to obtain a homogeneous concentration of salt and to maintain standard DO concentration. The replacement of water from each container was done every single day by siphoning the bottom of the each container at 96 h rearing period.

Feeding and threat response

Feed was applied to the fish after 6 h of stocking. The fish larvae were fed with meshed egg yolk several times in a day. The fish fry were fed with commercial fish feed at 6-10% of total body weight four times in a day. Fingerling were fed with commercial fish feed at 3-5% of total body weight two times in a day.

Feeding response was determined for each ontogenetic stages by the absence or presence and quantity of leftovers in the bottom of the glass tanks. Very High

Appetite (VHA) indicates no leftover food whereas Low Appetite (LA) indicates almost all food is leftover.

Various behavioral anomalies were observed in *H. fossilis* larvae, fry and fingerling at different concentration of salinity. Threat response by erratic or restlessness or hyper activeness, aggressive or normal behavioral patterns displayed by the fish when touched with a glass rod were used as baseline for data collection.

Water Quality Monitoring

Temperatures, dissolved oxygen (DO) and pH was monitored before and during the experiment. Water temperatures, dissolved oxygen and pH levels were measured using the portable digital Celsius thermometer and portable digital dissolve oxygen (DO) meter (Model: Lutron, PDO-519) and a portable digital pH tester (Model: HI 98107 pHep) respectively at each containers.

Calculation

Concentration of the chemical transformed into log concentration (X) and the percentages mortality were converted into probit mortality (Y) according to the method of Doudoroff *et al.,* (1951). Then, Empirical probit value (Y) and log concentration of salinity (X) were plotted in Y and X axis, respectively. Linear regression equation is $Y = bX + a$ (where 'b' is the slope and the value obtained as $b = \sum xy/\sum x^2$ ($y=Y- \bar{Y}$, $x= X- \bar{X}$); 'a' is the intercept and value of 'a' obtained as $a = \bar{Y}-b\bar{X}$. By substituting value of a, b regression equations were obtained separately for 24, 48, 72 and 96 h dose mortality responses. To draw straight line through the data points, x and y value of the equation were substituted to obtain four x, y coordinates. Log concentration of salinity corresponding to empirical probit value 5 was determined as log LC_{50} value which was converted to LC_{50} value. Thus, 24 h, 48 h, 72 h and 96 h LC_{50} of salinity for *H. fossilis* was determined.

Statistical Analysis

All the structured designs and data were analyzed using a one-way ANOVA. This included significant results ($p<0.05$) were taken as rejection of the null hypothesis which showed significance differences between the treatments. These results are displayed as superscripts against each respective value. All statistical analyses were carried out using Microsoft excel program (version 2013) and statistical software Statistix 10.

RESULTS AND DISCUSSION

Probit value of mortality (%) of larvae

Dose and mortality responses of the experimental fish *H. fossilis* larvae after different concentration of saline water exposure are shown in Table 1 and regression curve for 24 h, 48 h, 72 h and 96 h (Fig. 1A-1D).

No mortality was observed in case of control (0 ppt salinity) group, whereas rest of the treatments (T_2, T_3, T_4, T_5, T_6 and T_7) showed varying level of cumulative mortality depending on the salinity and the period of exposure. Treatment T_2 (2 ppt salinity) showed 7% mortality after 24 h of exposure that rose to 33% by the end of 96 h. Similar trend was found in case of treatment T_3 which experienced 7% mortality of fishes at 9 h of exposure, and within 96 h of exposure the mortality rate increased to 67%. On the other hand, 100% mortality of fishes was observed in treatment T_4, T_5, and T_6 within 84 h, 36 h and 3h of exposure, respectively. Furthermore, treatment T_7, having the highest salinity level of 12 ppt, resulted in 100% fish mortality within 0.5 h of exposure. Overall, the mortality rate increased with increasing salinity as well as with increasing exposure period. LC_{50} value of 5 days larvae were calculated based on the dose mortality Table 1. Salinity concentration that caused 0% and 100% mortality were not considered for calculation as 0% and 100% mortality has no probit value. From the linear regression curve corresponding point for the probit mortality 5 (log LC_{50} of salinity) was found 0.82, 0.61, 0.47 and 0.45 for 24 h, 48 h, 72 h and 96 h, respectively. Therefore, 24, 48, 72 and 96 h LC_{50} was determined as 6.61 ppt, 4.07 ppt, 2.95 ppt and 2.82 ppt respectively (Fig. 1: 1A-1D).

Probit value of mortality (%) of fry

Dose and mortality responses of the experimental fish *H. fossilis* fry during exposure at different concentration of saline water are shown in Table 2 and regression curve for 24 h, 48 h, 72 h and 96 h (Fig. 2A-2D).

No mortality was found in treatment T_1 (control group). Whereas, only 7% mortality occurred in treatment T_2 (2 ppt salinity) within the experimental period and 20% mortality was observed in T_3 (4 ppt salinity) and T_4 (6 ppt salinity), respectively at the last day of experiment. In T_5 and T_6 which have 8 ppt and 10 ppt salinity, 47% and 80% mortality was observed at the end, respectively. In case of T_7 having highest salinity level, 100% mortality was observed within the 36 h of exposure period. The mortality of fry was increased with increasing salinity concentration and exposure time. LC_{50} value of 21 days fry were calculated based on the dose mortality Table 2. Salinity concentration that caused 0% and 100% mortality were not considered for calculation as 0% and 100% mortality has no probit value. From the linear regression curve corresponding point for the probit mortality 50% (log LC_{50} of salinity) was found 1.03, 0.98, 0.93 and 0.88 for 24 h, 48 h, 72 h and 96 h, respectively. Therefore, 24, 48, 72 and 96 h LC_{50} was determined as 10.71 ppt, 9.549 ppt, 8.511 ppt and 7.585 ppt respectively (Fig. 2: 2A-2D).

Probit value of mortality (%) of fingerling

Dose and mortality responses of the experimental fish *H. fossilis* fingerling during exposure at different concentration of saline water are shown in Table 3 and regression curve for 24 h, 48 h, 72 h and 96 h (Fig. 3A-3D).

There was no death either in control (0 ppt) group of fish or in fish groups exposed up to T_3 (4 ppt salinity) during the experimental period. 7% mortality occurred at T_4 (6 ppt salinity) and T_5 (8 ppt salinity) within 60 h and 24 h of exposure, respectively and both group of fish showed 7% mortality by 96 h of exposure. Whereas, in T_6 which have 10 ppt salinity, 7% mortality was recorded within 24 h of exposure and it rose up to 40% at the end of the experiment. On the other hand, T_7 (12 ppt) with highest concentration of salinity started with 7% mortality within 12 h of exposure

and it increased up to 87% within the exposure time. The survival rate significantly decreased when salinity increased. LC_{50} value of 45 days fingerling was calculated based on the dose mortality Table 3. Salinity concentration that caused 0% and 100% mortality were not considered for calculation as 0% and 100% mortality has no probit value. From the linear regression curve corresponding point for the probit mortality 50% (log LC_{50} of salinity) was found 1.35, 1.1, 1.06 and 1.0 for 24 h, 48 h, 72 h and 96 h, respectively. Therefore, 24, 48, 72 and 96 h LC_{50} was determined as 22.387 ppt, 12.589 ppt, 11.481 ppt and 10.00 ppt respectively (Fig. 3A-3D).

Effect of salinity on feeding response of H. fossilis larvae, fry and fingerling

H. fossilis showed very high appetitive (VHA) behavior to food at all the three stages (larvae, fry and fingerling) between treatment T_1 (control) and treatment T_2 (2 ppt salinity) during 96 h exposure time. The fry and the fingerling showed high appetitive (HA) behavior to food up to treatment T_4 (6 ppt salinity) and treatment T_5 (8 ppt salinity), respectively. Moderate appetitive (MA) behavior was observed at treatment T_3 (4 ppt salinity) and treatment T_4 (6 ppt salinity) for larvae, treatment T_5 and treatment T_6 (10 ppt salinity) for fry and treatment T_6 and treatment T_7 (12 ppt salinity) for fingerling. From then on, low appetitive (LA) behavior followed by death occurred at treatment T_5, treatment T_6 and treatment T_7 for larvae and at treatment T_7 in case of fry (Table 4).

Effect of salinity on threat response of H. fossilis larvae, fry and fingerling

Various behavioral anomalies were observed in *H. fossilis* larvae, fry and fingerling at different concentration of salinity. Intoxication and death following treatment with salt and different behavior associated with them varied with the rate of application and the time of exposure. Behavioral anomalies in fish observed during the exposure are briefly summarized in Table 5. The fish exhibited a normal response to threat at all stages between treatment T_1 (control) and treatment T_2 (2 ppt salinity) during the exposure time. The fry showed normal response up to treatment T_4 (6 ppt salinity), and the fingerling showed normal response up to treatment T_5 (8 ppt salinity). Moderate response was found in 2nd day and 3rd day at treatment T_3 (4 ppt salinity) for larvae, treatment T_5 and treatment T_6 (10 ppt salinity) for fry and treatment T_6 and treatment T_7 (12 ppt salinity) for fingerling. From then on, fish became hyperactive and sequentially mortality occurred at treatment T_5, treatment T_6 and treatment T_7 for larvae and at treatment T_7 in case of fry. The restlessness or hyper-activeness or erratic behavior in high salinities indicates fast rate at which the fish were approaching their tolerance limits and loss of water to external medium from the body. In case of *H. fossilis* from this research normal response was found up to 4 ppt salinity for larvae, 6 ppt salinity for fry and 8 ppt salinity for fingerling, respectively and then hyper-activeness or erratic behavior or death shown at 6 to 12 ppt salinity. Such changes with increased salinity are indication that the salinities were near or outside the tolerance limits of the fish. Moderate and normal responses represented the near and far tolerance limits respectively.

Water quality parameters

The water quality variables in the test container were not significantly different (P>0.05) among treatments for the three life stages. The mean range of water temperature, DO and pH of larvae, fry and fingerling ranges between 27.35- 28.93°C, 6.40-7.05 mg L^{-1} and 7.14-7.81, respectively among the treatments.

The ability of fish to adapt to any medium depends on its ability to maintain body osmoregulation. The body fluids of freshwater fishes are hypertonic in comparison to external medium and they osmoregulate by producing copious urine as well as by active uptake of ions through gills. Sudden death results when freshwater fishes are exposed to normal seawater. This result was supported by Britz and Hecht (1989) who studied the effect of salinity on growth and survival of *C. gariepinus* larvae. They found no significant differences in mortality between 0 and 5 ppt salinity. At 7.5 ppt mortality rate was higher and at 10 ppt 100% fish were died within 48 h.

The study was carried out to find the range of salinity tolerance at different ontogenetic stages (larvae, fry and fingerling) of *H. fossilis* from 0 ppt to 12 ppt. The overall mortality rate of larvae for *H. fossilis* increased with increasing salinity as well as with increasing exposure period. 67% larvae were unable to tolerate extreme salinity conditions (4 ppt), indicating that salinity greater than 2 ppt would probably represent a stress factor for the larvae signifying that the freshwater or estuarine environment fitted best for larval stages, at least under laboratory conditions and excluding other variables. This might be due to changes in ontogeny and salinity which influenced the buoyancy and survival of larvae (Spencer *et al.,* 2020). Similarly, lower survival was observed in newly-hatched Nile tilapia *O. niloticus* at higher salinities (15, 20 and 25 g L^{-1}) compared to lower salinities (0 and 7.5 g L^{-1}) during 9 days post hatched period (Fridman *et al.,* 2012). In the present study, estimated 24, 48, 72 and 96 h LC_{50} values of *H. fossilis* larvae were 6.61, 4.07, 2.95 and 2.82 ppt, respectively. Garcia *et al.,*(1999) conducted a study on survival and growth of Bighead Carp (*A. nobilis*) fry exposed to low salinities and reported that final overall survival of 11 day old fry declined markedly following exposure to 4 ppt and above, resulting in MLS (Median Lethal Salinity) of 2.3 ppt indicating MLS of bighead carp fry exposed to various salinities increased with age. The result of their findings corroborated with the study of this experiment. Salinity influence on the early stages of the African catfish was studied by Britz and Hecht (1989). They estimated the value of 24, 48, and 96 h MLS of African catfish (*C. gariepinus*) were 12 ppt, 10 ppt and 8 ppt. Amin *et al.,* (2016) also reported the MLS of *B. gonionotus* was 12.9 ppt. Thus, variation might be due to species difference and their physiological causes.

In this experiment, estimated 24, 48, 72 and 96 h LC_{50} values of *H. fossilis* fry were 10.71, 9.54, 8.51 and 7.58 ppt, respectively. Gabriel *et al.*, (2012) also determined the 48 h MLS for 14 and 21 day old were 4.52 and 4.79 ppt and 96 h MLS were 4.40 and 4.43 ppt for the 14 day-old fry and the 21 day old fry, respectively.

In this study, *H. fossilis* fry can tolerate salinity up to 6 ppt which is similar to the findings of Garcia *et al.*, (1999), who observed that the 18 day old bighead carp fry was able to survive in 4 ppt salinity and in case of 35 day

old fry it was 6 ppt (Garcia *et al.,* 1999). Whereas, *B. gonionotus* fry can tolerate salinity up to 10 ppt (Faizul and Christianus, 2013) and young catfish (*C. lazera*) were able to tolerate 9.75 ppt (Chervinski, 1984) which is higher than the present findings. This difference in tolerance limit might be due to the variation in fish species and their age.

From the result of the experiment, estimated 24, 48, 72 and 96 h LC_{50} values of *H. fossilis* fingerling were 22.38 ppt, 12.58 ppt, 11.48 ppt and 10 ppt. Akther *et al.* (2009) calculated a 24, 48, 72 and 96 h LC_{50} value of 12.74, 12.46, 12.36 and 12.27 ppt for Thai silver barb, *B. gonionotus* which supports the present findings. A 96 h LC_{50} value of 13 ppt was recorded for adult *C. punctata* and 12.52 ppt for fingerling of *C. batrachus* (Sarma *et al.,* 2013). Dubey *et al.,* (2015) investigated the effect of salinity on growth and survival of *A. testudineus* for assessing their culture potential in brackish water. The estimated median lethal salinity concentration of 96 h for *A. testudineus* (11.74 g) was 18.86 ppt. He also studied 96 h median lethal salinity for *A. mola* which was found to be 6.20 ppt whereas for *P. ticto* it was 6.12 ppt (Dubey *et al.,* 2014). The variance in results could be due to the differences in fish size and age and also due to species variation.

The survival of fingerlings was 100% up to 4 ppt salinity and it was 93% in 6 and 8 ppt, respectively during the 96 h of exposure time. This is an indication that the fingerlings were able to regulate their body physiology within this salinity regime and it is supplemented by the findings of Islam *et al.,* (2014), recorded 100% survival rate up to 6% salinities in rohu fingerlings. Similar result was also reported in case of common carp fingerling and climbing perch *A. testudineus* (Dubey *et. al.,* 2015; Mangat and Hundal, 2014). Ghosh *et al.* (1973) reported that *C. catla* and *L. rohita* fry and fingerlings could tolerate 8 ppt salinity without mortality but survival gradually decreased with increase of salinity. Complete mortality was observed in these species at 15 ppt salinity (Ghosh *et al.,* 1973). Similarly, Mansuri *et al*. (1979) observed that *C. punctatus* could thrive well in 10% seawater for indefinite period and mortality started beyond 30% seawater. Researchers have recorded 71-90% mortality in 24 h under 15% salinity in grass carp fingerlings (Kilambi and Zdinak, 1980) while Crivelli (1981) has reported that the common carp occurred in brackish-water marshes with salinities up to 14 ppt in southern France. In the present study, at 12 ppt salinity, mortality was observed from very first day and 87% mortality within 96 h was recorded. Salinity stress in freshwater fish primarily affects the gills, as the major organ involved both in osmoregulation and waste nitrogen excretion (Nikolsky, 1963). High salinity has been known to display a highly disrupted epithelium with a diffuse edema of both the primary and the secondary lamellae (Holliday and Jones, 1967) and this could be the reason why 87% mortality of fish fingerlings in 12 ppt salinity was observed.

Mangat and Hundal (2014) observed a high appetitive behavior of *C. carpio* fingerlings between 0 to 3 ppt salinity. High appetitive behaviors was also recorded between 0 to 4 ppt salinity by Islam et al., (2014) for *L. rohita* fingerling. In this study, high appetitive behavior of *H. fossilis* was recorded between 0 to 4 ppt salinity and sequentially lowered and death occurred at 12 ppt salinity. The high appetitive behavior displayed by the fish towards food is an indication that fish body metabolism can still be maintained or regulated in these salinities, while low appetite is an indication of near or total body metabolic break down. Observation made by scientists about *O. niloticus* showed high appetitive behavior between 0 to 7 ppt salinities (Resendez, 1981). Common carp, *C. carpio* showed high resistant, better feeding rate and as well as growth up to 6‰ salinity level (Mangat and Hundal, 2014).

Fish move to preferred position in salinity gradient, to indicate salinity preferences in choice situation (Baggerman, 1959; McInerney, 1964). Islam *et al.,* (2014) found normal response up to 6 ppt salinity and then hyper-activeness or erratic behavior or death shown at 8 to 12 ppt salinity for *L. rohita* fingerling which is similar to the present study. Amin *et al.,* (2016) also noted similar behavioral stress responses after exposing fish in higher salinities. Dubey *et al.* (2014) also observed normal response between 0 to 4 ppt salinity in case of *A. mola* and *P. ticto*. Hoar and Randal (1969) based the survival of fish on a combination of tissue tolerance and regulation, higher osmoregulatory cost at higher salinity could make fish to develop body lesions which covered 25% of their body surface.

Table 1: Dose mortality responses of *H. fossilis* larvae at different salinity regimes within 96-h exposure time

Treatment / Conc. of salinity (ppt)	No. of fish exposed	Log Conc. (X)	24 h		48 h		72 h		96 h	
			% Mortality	Probit Mortality (Y_1)	% Mortality	Probit Mortality (Y_2)	% Mortality	Probit Mortality (Y_3)	% Mortality	Probit Mortality (Y_4)
T_1 (0)	15	0	0	0	0	0	0	0	0	0
T_2 (2)	15	0.301	7	3.53	20	4.16	33	4.56	33	4.56
T_3 (4)	15	0.602	7	3.53	33	4.56	47	4.92	67	5.44
T_4 (6)	15	0.778	47	4.92	80	5.84	93	6.48	LD	
T_5 (8)	15	0.903	73	5.61	LD		LD		LD	
T_6 (10)	15	1	LD		LD		LD		LD	
T_7 (12)	15	1.079	LD		LD		LD		LD	

LD = Lethal Dose (100% mortality)

Table 2: Dose mortality responses of *H. fossilis* fry at different salinity regimes within 96-h exposure time

Treatment / Conc. of salinity (ppt)	No. of fish exposed	Log Conc. (X)	24 h		48 h		72 h		96 h	
			% Mortality	Probit Mortality (Y_1)	% Mortality	Probit Mortality (Y_2)	% Mortality	Probit Mortality (Y_3)	% Mortality	Probit Mortality (Y_4)
T_1 (0)	15	0	0	0	0	0	0	0	0	0
T_2 (2)	15	0.301	0	0	0	0	0	0	7	3.53
T_3 (4)	15	0.602	7	3.53	7	3.53	13	3.87	20	4.16
T_4 (6)	15	0.778	7	3.53	7	3.53	13	3.87	20	4.16
T_5 (8)	15	0.903	13	3.87	40	4.75	47	4.92	47	4.92
T_6 (10)	15	1	27	4.39	60	5.25	67	5.44	80	5.84
T_7 (12)	15	1.079	87	6.13	LD		LD		LD	

LD = Lethal Dose (100% mortality)

Table 3: Dose mortality responses of *H. fossilis* fingerling at different salinity regimes within 96-h exposure time

Treatment / Conc. of salinity (ppt)	No. of fish exposed	Log Conc. (X)	24 h		48 h		72 h		96 h	
			% Mortality	Probit Mortality (Y_1)	% Mortality	Probit Mortality (Y_2)	% Mortality	Probit Mortality (Y_3)	% Mortality	Probit Mortality (Y_4)
T_1 (0)	15	0	0	0	0	0	0	0	0	0
T_2 (2)	15	0.30	0	0	0	0	0	0	0	0
T_3 (4)	15	0.60	0	0	0	0	0	0	0	0
T_4 (6)	15	0.78	0	0	0	0	7	3.53	7	3.53
T_5 (8)	15	0.90	7	3.53	7	3.53	7	3.53	7	3.53
T_6 (10)	15	1	7	3.53	27	4.39	27	4.39	40	4.75
T_7 (12)	15	1.08	20	4.16	40	4.75	67	5.44	87	6.13

Table 4: Summary of daily feeding response of *H. fossilis* larvae, fry and fingerling in different salinity regimes

Treatment (Conc.)	Time duration (days)											
	Larvae				Fry				Fingerling			
	1	2	3	4	1	2	3	4	1	2	3	4
T_1 (0 ppt)	VHA	VHA	VHA	VHA	VHA	VHA	VHA	VHA	VHA	VHA	VHA	VHA
T_2 (2 ppt)	VHA	VHA	HA	HA	VHA	VHA	VHA	VHA	VHA	VHA	VHA	VHA
T_3 (4 ppt)	HA	HA	MA	LA	VHA	VHA	HA	HA	VHA	VHA	VHA	VHA
T_4 (6 ppt)	MA	LA	LA	D	VHA	VHA	HA	HA	VHA	VHA	HA	HA
T_5 (8 ppt)	LA	D	D	D	HA	MA	MA	MA	VHA	HA	HA	HA
T_6 (10 ppt)	D	D	D	D	HA	MA	MA	LA	HA	MA	MA	MA
T_7 (12 ppt)	D	D	D	D	LA	D	D	D	HA	MA	MA	LA

VHA=Very High Appetite, HA= High Appetite, MA=Moderate Appetite, LA=Low Appetite, D=Death (100% mortality)

Table 5: Threat response of *H. fossilis* in different salinity regimes at different duration

Treatment	Time duration (days)											
	Larvae				Fry				Fingerling			
	1	2	3	4	1	2	3	4	1	2	3	4
T_1 (0 ppt)	N	N	N	N	N	N	N	N	N	N	N	N
T_2 (2 ppt)	N	N	N	N	N	N	N	N	N	N	N	N
T_3 (4 ppt)	N	M	M	H	N	N	N	N	N	N	N	N
T_4 (6 ppt)	M	H	H	D	N	N	N	N	N	N	N	N
T_5 (8 ppt)	H	D	D	D	N	M	M	M	N	N	N	N
T_6 (10 ppt)	D	D	D	D	N	M	M	H	N	M	M	M
T_7 (12 ppt)	D	D	D	D	H	D	D	D	N	M	M	H

N=Normal Response, M=Moderate Response, H=Hyperactive, D=Death (100% mortality)

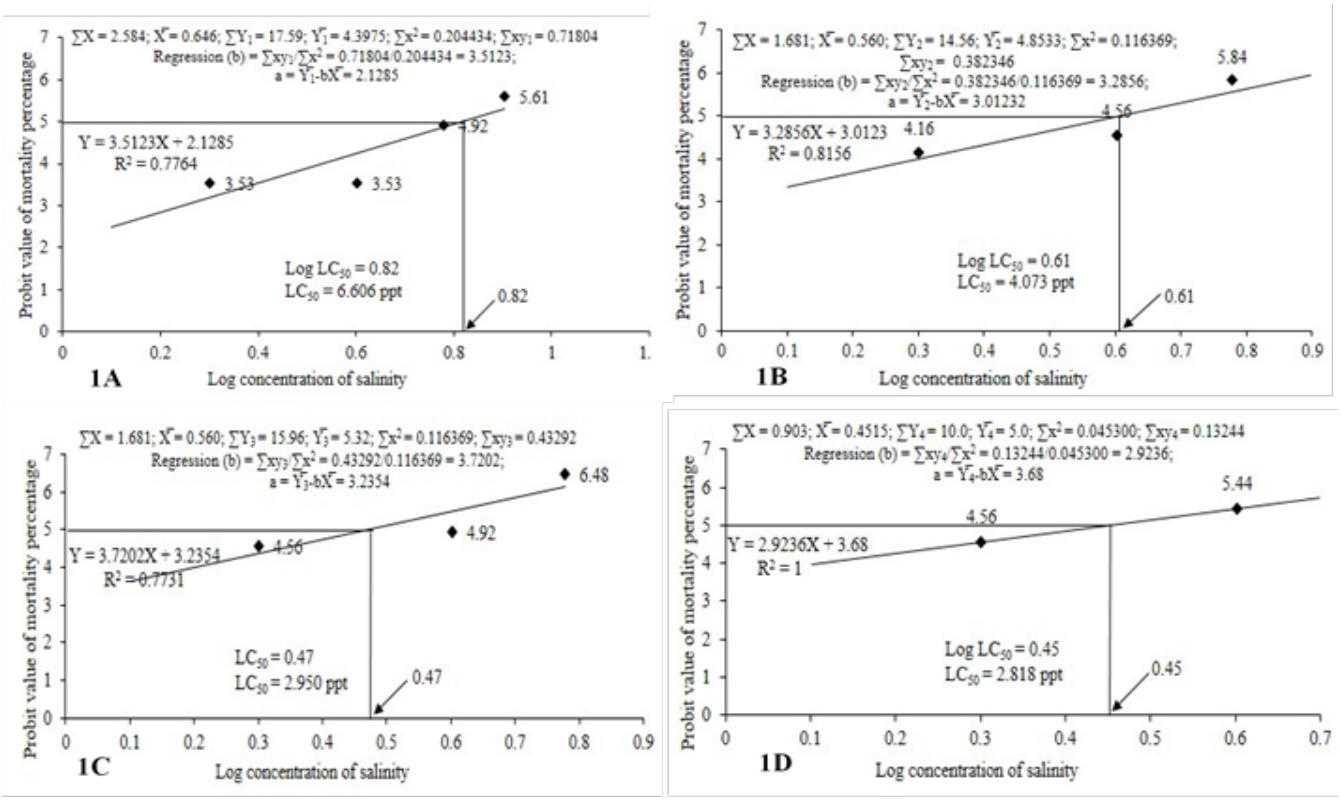

Fig. 1: Linear regression curve of dose and mortality response of larvae of *H. fossilis* after exposure to salinity. 1A- 24 h; 1B- 48 h; 1C- 72 h and 1D- 96 h.

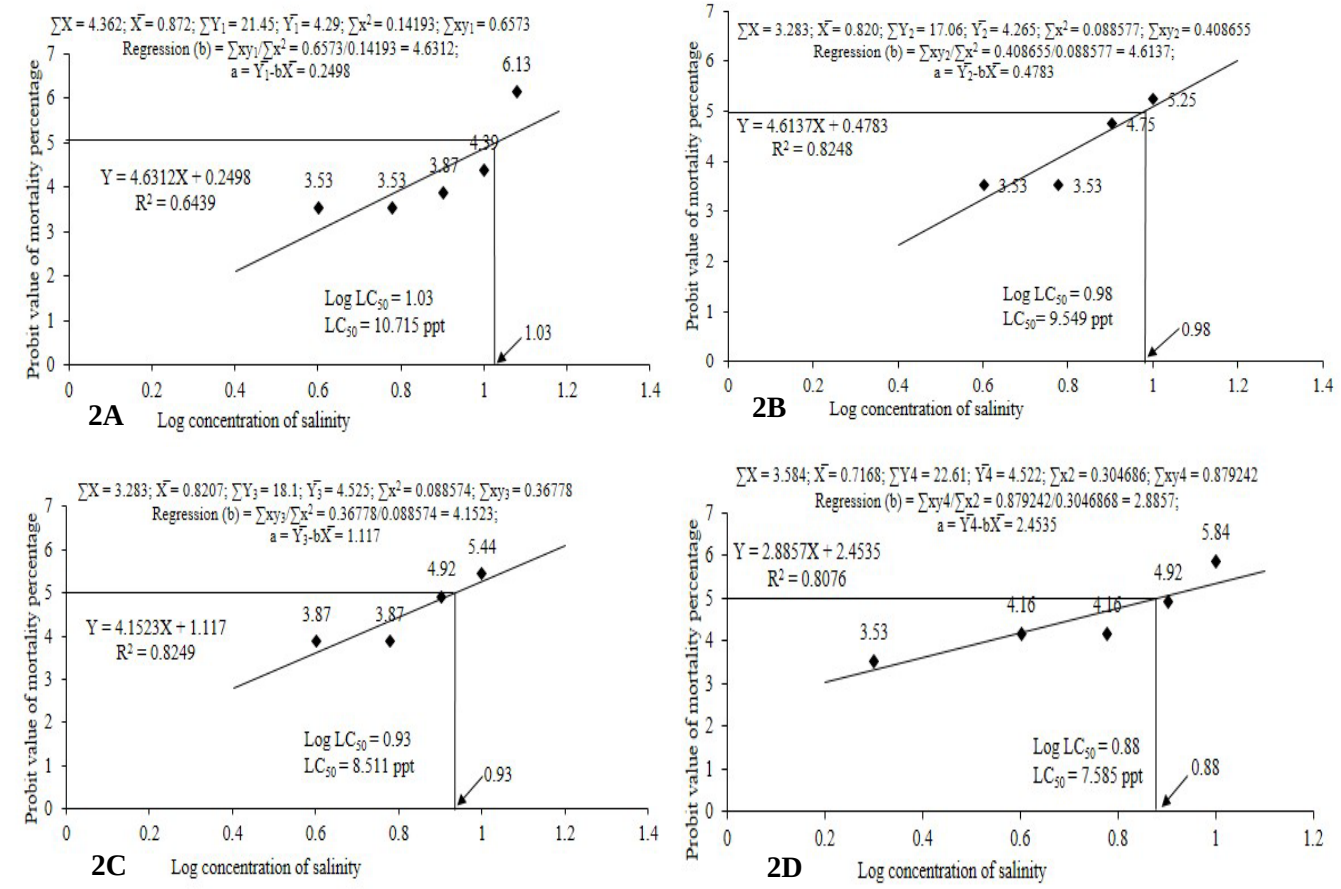

Fig. 2: Linear regression curve of dose and mortality response of fry of *H. fossilis* after exposure to salinity. 2A- 24 h; 2B- 48 h; 2C- 72 h and 2D- 96 h.

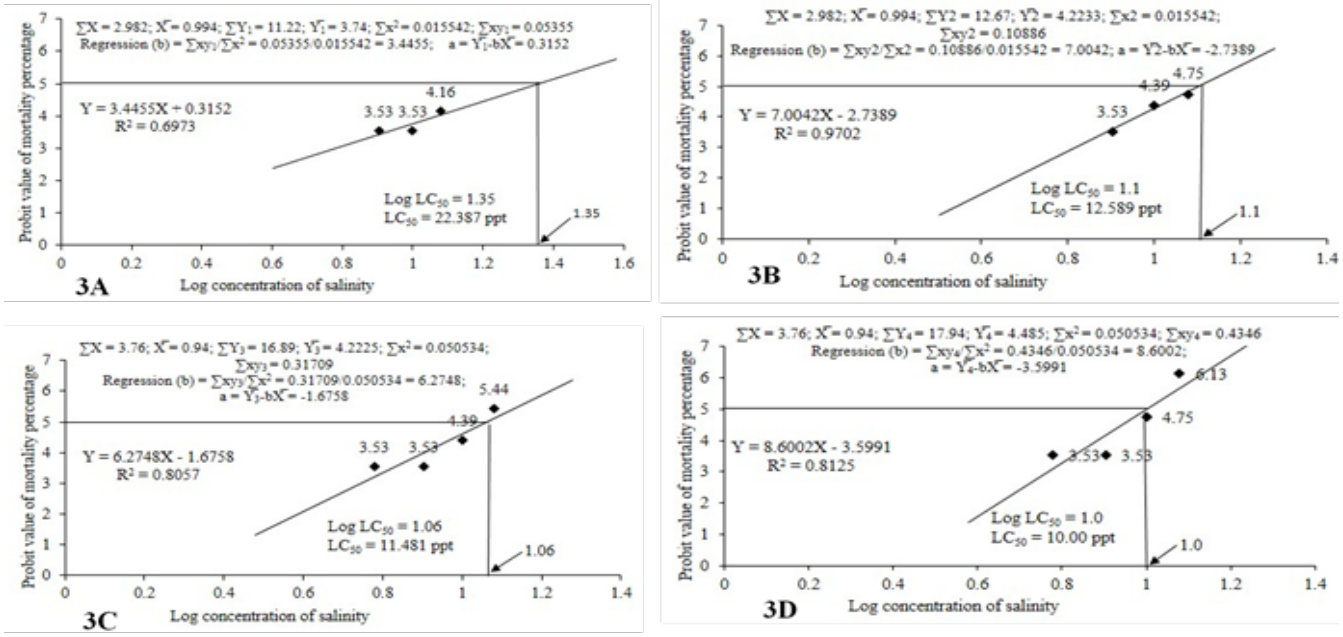

Fig. 3: Linear regression curve of dose and mortality response of fingerling of *H. fossilis* after exposure to salinity. 3A- 24 h; 3B- 48 h; 3C- 72 h and 3D- 96 h.

CONCLUSION

The stinging catfish *H. fossilis* is a very hardy and commercially important indigenous fish species in Bangladesh. The present study was carried out to determine the salinity tolerance limit and to observe the effects of salinity on survival, feeding response and behavioral responses of *H. fossilis* at their different life stages (larvae, fry and fingerling). From the result of this experiment 24, 48, 72 and 96 h LC_{50} values of *H. fossilis* were 6.61, 4.07, 2.95 and 2.82 ppt, respectively for larvae. Whereas 24, 48, 72 and 96 h LC_{50} values were 10.71, 9.54, 8.51 and 7.58 ppt, respectively for fry. On the other hand, 24, 48, 72 and 96 h LC_{50} values for fingerling were 22.38, 12.58, 11.48 and 10 ppt, respectively. *H. fossilis* 5 days larvae could not survive more than 2 ppt salinity, whereas 21 days fry could survive up to 6 ppt salinity and 45 days fingerling up to 8 ppt salinity. The results of the present experiment indicated that salinity plays a significant role for the culture of *H. fossilis* and the species can adapt to gradual increase of salinity and fry and fingerling showed satisfactory survival at salinity range of 2 to 6 ppt. In view of the current and future climate variables, more coastal areas of Bangladesh are going to be vulnerable to brackish water inundation. Under such scenario, *H. fossilis* can be considered as an ideal species where brackishwater intrusion is frequent phenomenon.

ACKNOWLEDGEMENT

This work was funded by a grant of Ministry of Science and Technology, Government of the People's Republic of Bangladesh.

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