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Median Lethal Salinity ($MLS_{96\ h}$) of Two Small Indigenous Fish Species *Amblypharyngodon mola* and *Pethia ticto* from Indian Sundarban

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Abstract

Amblypharyngodon mola and *Pethia ticto* are small indigenous fish species belonging to family cyprinidae and considered as primary freshwater fish widely distributed in Indian subcontinent including Sundarban region. The 96-h median lethal salinity ($MLS_{96\ h}$) level of these fish was found out by exposing to saline water (0-10 ppt) in direct transfer method. The 96-h median lethal salinity for *A. mola* was found to be 6.20 ppt with 95% confidence intervals of 4.38-7.09 ppt where as for *P. ticto* it was 6.12 with 95% confidence intervals of 3.67-7.07 ppt. The Probit showed that at 6.12-6.20 ppt, 50% of the both test species shows sensitivity to salinity that leads to mortality. The regression analysis indicated that the mortality rate is positively correlated with salinity concentration having a regression coefficient close to 1.0 in each case. Various levels of external stress responses were noticed at 8 and 10 ppt salinity. The study suggests that these fish can potentially be used as a candidate species for aquaculture in slight brackishwater areas of Sundarban. However, further studies are required to understand the ecosystem based adaptation processes at higher salinity levels.

Keywords: *Amblypharyngodon mola*; Indian sundarban; Median lethal salinity; *Pethia ticto*; Salinity stress; SIS

Introduction

Amblypharyngodon mola and *Pethia* (*Puntius*) *ticto* are conspicuous member of small indigenous fish species (SIS) belonging to the family cyprinidae and considered as native primary freshwater fish species. These tropical Asian cyprinids are well distributed in Indian subcontinent including Sundarban region of West Bengal and commonly inhabit ponds, rivers, floodplain lakes, low lying swamps, marsh lands, canals, paddy fields and many other small water bodies etc [1-3]. SIS are considered as fish which grow to a length of approximately <25 cm at maturity [4]. *A. mola* and *P. ticto* have drawn special attention among more than 450 SIS available in India due to its food value, market demands and providing nutritional security through its high vitamin, mineral contents [5,6]. But the populations of these fish have seriously declined due to over exploitation and various ecological changes in its natural habitat setting.

Salinity is an important environmental factor which influences development, growth and distributions of many fish [7,8]. Salinity tolerance is an important consideration in the culture of marine and freshwater organisms providing information about basic culture requirements necessary for the species to thrive in captivity as well as potential applications for assessing the distribution of fish and their impact on ecosystems [9,10].

The world's largest luxuriant mangrove chunk, the Sundarban (21° 30′ to 22° 40′ N, 88° 05′ to 89° 55′ E) at the mouth of Ganga-Brahmaputra-Meghna basin, is a unique bioclimatic zone and genetically diverse eco-region on the apex of Bay of Bengal which is famous for finfish diversity. The freshwater flows from the rivers and the tidal ingress from the sea result in a gradient of salinity that varies both spatially and temporally. In general, the salinity is higher nearer the coast and the water is nearly fresh on the inland side boundary of the Sundarban [11]. Increasing salinity in rivers and wetlands is recognized as a serious environmental problem in all inhabited continents [12]. Likewise mangrove ecosystems are among the most threatened by the global climate changes, particularly the sea level rise [13]. This UNESCO declared World Heritage Site is prominently witnessing climate induced changes since last decades. Many areas of the regions are prone to saline water inundation and subject to environmental hazards during extreme weather events like cyclones and storm surges. During the severe tropical cyclone *Aila* in 2009, a large proportion of freshwater areas of sundarban were completely submerged by brackish water. Due to this severe cyclonic storm, high energy tidal surges brought changes in environmental parameters, specially the average water salinity from 13.64 ± 6.24 ppt to 17.08 ± 8.03 ppt with an increase of 25.2% [14]. Due to salinity intrusion in freshwater aquaculture areas, many freshwater fish species are subjected to severe stress and threat due to their inability to cope up with such extreme conditions. In this background there is an urgent need to ascertain the salinity tolerance limit of some freshwater fishes inhibiting in this region.

The maximum salinity tolerance of a fish may not be exactly defined exclusively by laboratory observations because fish distribution may be influenced by other factors [15]. Acute toxicity tests are generally characterized by the median lethal concentration (LC50) over a specific period of time, generally 24 to 96 h when mortality is the end point. Unlike field observations, experimental determinations of salinity tolerance establish a causal link between salinity and mortality [16].The most common and simple experimental measure of salinity tolerance is the acute LC50 (median lethal concentration). The Median Lethal Salinity-96 h ($MLS_{96\ h}$) defined as the salinity at which survival falls

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to 50%, 96 h following direct transfer from freshwater to various test salinities [17]. However, laboratory obtained acute salinity tolerance values have been reflected the maximum salinity that fish species inhabit in the environment and used to describe spatial zonation and assemblage patterns [15,18]. Additionally results of acute salinity tolerance tests have been used to predict the likelihood for range expansion of non-indigenous species in estuarine environments [19].

Some aspects of bio-ecology of *A. mola* and *P. ticto* have been studied by several workers [20-24]. Various aspects of culture technologies of *A. mola* are also known [25-27]. Few information on the salinity tolerance of Indian major carps were also available [28,29] but salinity tolerance of *A. mola* and *P. ticto* hitherto not been touched. In continuation to the fact, an attempt has been made in the present study to determine Median Lethal Salinity ($MLS_{96\ h}$) for *A. mola* and *P. ticto* inhibiting in Indian Sundarban region.

Materials and Methods

Test animals and study site

Healthy and active sub-adult/adult fish were collected from the freshwater floodplain area (22°49'55.06" N and 88°07'35.7" E) (salinity constantly below 1 ppt) into oxygenated polythene bag (pH 7.5, alkalinity 100 ppm as $CaCO_3$, hardness 120 ppm as $CaCO_3$) and transported to NICRA Climate Resilient Aquaculture wet laboratory of WBUAFS located at S. D. Marine Biological Research Institute, Western fringe of Indian Sundarban mangrove eco-region. Upon arrival in the wet laboratory, the fish were acclimatized separately in 50L holding tank filled with freshwater for a period of 2 days at 29.5 ± 1°C temperature and 6.5 ± 0.75 ppm dissolved oxygen before commencement of experiment. The mean weight and length of *A. mola* was 1.7 ± 0.25 g and 3.8 ± 0.46 cm and *P. ticto* was 8.5 ± 0.50 g and 8.25 ± 0.27 cm (random sub-sample of 40 fish each) respectively. The fish were fed daily *ad libitum* with crushed pelleted feed during acclimation period.

Median lethal salinity test

A static nonrenewable acute toxicity bioassay was conducted according to standard methods of APHA [30] using the direct transfer method [16] following exposure of 96 h. Desired salinities were achieved by appropriately mixing dechlorinated tap water with natural seawater (~ 32 ppt) of Bay of Bengal. 20 numbers of uniform sized fish were randomly selected and directly transferred from 0 ppt to 2, 4, 6, 8, 10 ppt salinity and observed for 4 days. Stocking density maintained was 20 fish/tank. Experiment was conducted in 200 L identical FRP tanks in which 150L water volume was maintained. For this short-term test, a standard photoperiod of 16 h light: 8 h dark was mentioned and terminated feeding before commencement of trial. For carrying out each experiment, two replicates were run simultaneously following a Completely Randomized Design (CRD). Records of mortality were made at logarithmic time intervals (24, 48, 72, and 96 h) from the starting of the test. However, in addition to fixed time observations, several inspections were made in between these periods and dead fish were removed as soon as they were found. Fish were considered as dead when respiratory movement of the opercula stopped and there was no response to touch.

Water quality parameters

Water quality parameters of the experimental tanks were monitored daily. Temperature, pH, dissolved oxygen and salinity were determined directly by digital water analysis instrument (HANNA, HI 9828, Germany) while Ammonia-Nitrogen and Nitrate-Nitrogen were measured using HACH Spectrophotometer (DR 2800, Germany). Alkalinity and hardness were measured as per APHA [30].

Behavior observation

Various behavioral anomalies were noted in test animals when exposed to the saline water. Behavioral (stress) responses of fish such as opercula activity, convulsions, equilibrium status, hyperactivity, swimming etc were observed [31,32]. Signs like aggression, jumping, frequent surface bottom movements, sluggish and swirling movements, erratic swimming etc were also documented in first 24 hours.

Analysis of experimental data

The Median Lethal Salinity for 96 h ($MLS_{96\ h}$) was calculated by Probit method [33] by pooling mortality data from replicates within treatments and considered significantly different when the corresponding 95% confidence intervals did not overlap by using statistical software SPSS 10.0 for Windows (SPSS Inc. Chicago, IL USA). On the other hand, Analysis of variance (ANOVA) was employed to compare survival rates (%) in different salinities with a Tukey's HSD means separation test. Survivorship curves were generated using statistical software Med calc® version 12.7.0 (MedCalc Software bvba, Ostend, Belgium).

Results

Mortality response and median lethal salinity

The Water quality conditions during MLS test of the experimental setting were within the suitable range for fish thriving and recommended ranges for toxicity tests conducted with fish [34,35]. The mean value obtained from each treatment in range of 0 ppt-10 ppt has been depicted in Table 1.

The cumulative mortality (%) at different time intervals and percentages of the survivors of *A. mola* and *P. ticto* exposed to different salinity concentration are presented in Table 2 and Table 3 respectively. It can be revealed that lethality effect started around 4 ppt with 10% mortality noted at 24 h for both the fish and 20% and 25% mortality was observed at 96 h of exposure respectively. At 6 ppt, 45% mortality was observed in *A. mola* within 72 h exposure, similarly 40%

Temperature (°C)	pH	Dissolved oxygen (ppm)	Ammonia-Nitrogen (ppm)	Nitrate-Nitrogen (ppm)	Alkalinity (ppm)	Hardness (ppm)
29.5-30.5	8.2-8.84	5.06-6.52	0.01-0.34	0.01-0.23	105.0-129.4	70.5-120.75

Table 1: Water quality conditions during MLS test of *Amblypharyngodon mola* and *Pethia ticto*. The mean value obtained from each treatment was presented in range of 0 ppt-10 ppt.

Exposed salinity (ppt)	Cumulative mortality (%) (Exposure time in hours)					Survival (%)
	1 h	24 h	48 h	72 h	96 h	
0	0	0	0	0	0	100^a
2	0	5	0	0	0	95^b
4	0	10	10	0	0	80^c
6	0	15	20	10	0	55^d
8	20	15	25	10	5	25^e
10	30	30	25	10	5	0^f
Superscripts like a, b, c… in **same columns** were significantly different (P<0.01) in Tukey's HSD mean separation test						

Table 2: Determinative test of salinity lethality in *Amblypharyngodon mola*: Cumulative mortality (%) at different salinity concentrations within 96-hours exposure time.

Exposed salinity (ppt)	Cumulative mortality (%) (Exposure time in hours)					Survival (%)
	1 h	24 h	48 h	72 h	96 h	
0	0	0	0	0	0	100[a]
2	0	5	0	0	0	95[b]
4	0	10	10	5	0	75[c]
6	0	15	15	10	0	60[d]
8	10	20	20	15	15	20[e]
10	25	30	30	10	5	0[f]
Superscripts like a, b, c… in **same columns** were significantly different (P<0.01) in Tukey's HSD mean separation test						

Table 3: Determinative test of salinity lethality in *Pethia ticto*: Cumulative mortality (%) at different salinity concentrations within 96-hours exposure time.

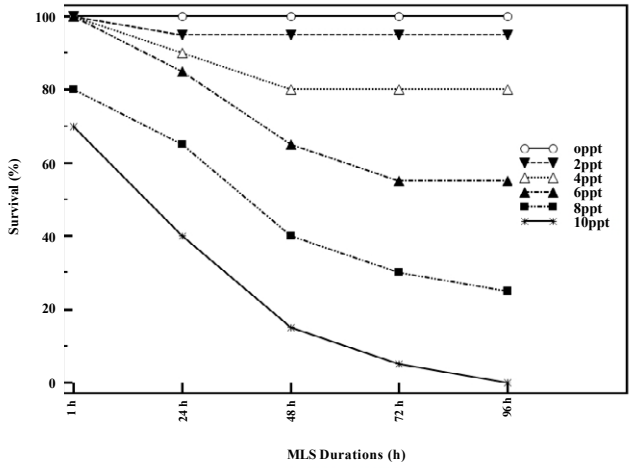

Figure 1: The survivorship curve of *Amblypharyngodon mola* in varied salinity.

mortality was documented at 6 ppt in *P. ticto* within the same time scale. At 8 ppt, 75% and 80% survival was noted for both *A. mola* and *P. ticto*; however 100% mortality occurred at 10 ppt for both the fish. In *A. mola*, 30% mortality was observed in 1h after direct transfer at 10 ppt and completed 100% within 96h exposure. Likewise in *P. ticto* 25% mortality was seen in 1h after direct transfer at 10 ppt with highest mortality at 24 h (30%) and reached 100% mortality at 96 h. In both fish, 100% survival rate was recorded in 0ppt followed by 95% in 2 ppt and survival rate significantly decreased when salinity increased (*A.mola*: $F_{5,12}$=1852.5; *P*<0.0001/*P. ticto*: $F_{5,12}$=4601.26; *P*<0.0001). The time (h) dependent survivorship curves for *A. mola* and *P. ticto* in varied salinities have been demonstrated in Figure 1 and Figure 2 respectively.

The estimated Median Lethal Salinity ($MLS_{96\ h}$) and confidence limits computed using Probit for *A. mola* and *P. ticto* are presented in Table 4 and Table 5. The 96 h-Median Lethal Salinity of *A. mola* (1.7 ± 0.25 g weight; 3.8 ± 0.46 cm length) is 6.20 ppt (LC50 Standard Error-0.42; Intercept-2.93, Beta-0.42) with confidence intervals (at 95%) of 4.38-7.09 ppt. In *P. ticto* (8.5 ± 0.50 g weight; 8.25 ± 0.27 cm length), 96 h-Median Lethal Salinity is 6.12 ppt (LC50 Standard Error-0.42; Intercept-2.43, Beta-0.42) with confidence intervals (at 95%) of 3.67-7.07 ppt. The Probit showed that at 6.12-6.20 ppt, 50% of the both test species shows sensitivity to salinity that leads to mortality. The statistical confidence of point estimate values other than 50% can be used to characterize toxicity (lethal salinity). The precision of the test results for a typical sigmoid cumulative distribution dose response curve for *A. mola* and *P. ticto* are depicted in Figure 3 and Figure 4 respectively. The result of regression analysis indicates that the mortality rate is positively correlated with the concentration having a regression coefficient close to one in each case. For *P. ticto*, the equation Y=2.01+0.07X defines a positive relationship between salinity (Y) and percentage mortality (X) with high positive correlation (R=0.98; *P*=0.001). The equation for *A. mola* is Y=2.04+0.08X with correlation R=0.99 (*P*=0.001).

Behavior

Various level of external stress responses were observed in test fish when exposed to >4 ppt salinity. Agitated behavior like hyper-activity, jumping, frequent surface bottom movements, erratic swimming was noticed in 8 ppt and 10 ppt salinity with fast opercula movement. Fish in 10 ppt gradually became lethargic, sluggish and swirling movements and tend to settle down on the bottom of the tank. Fish was found to swim back with occasional body convulsions at 8 ppt and 10 ppt salinity.

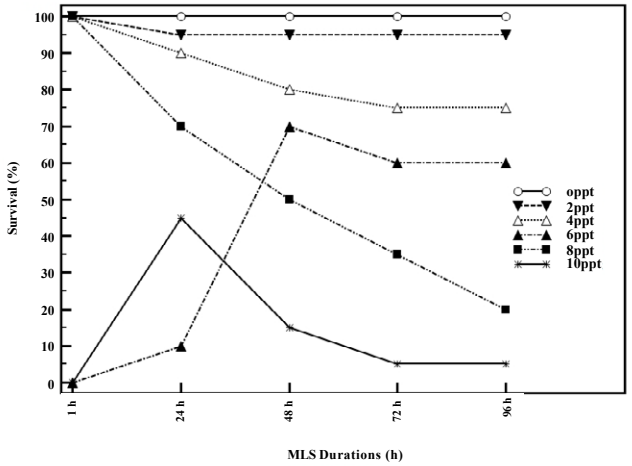

Figure 2: The survivorship curve of *Pethia ticto* in varied salinity.

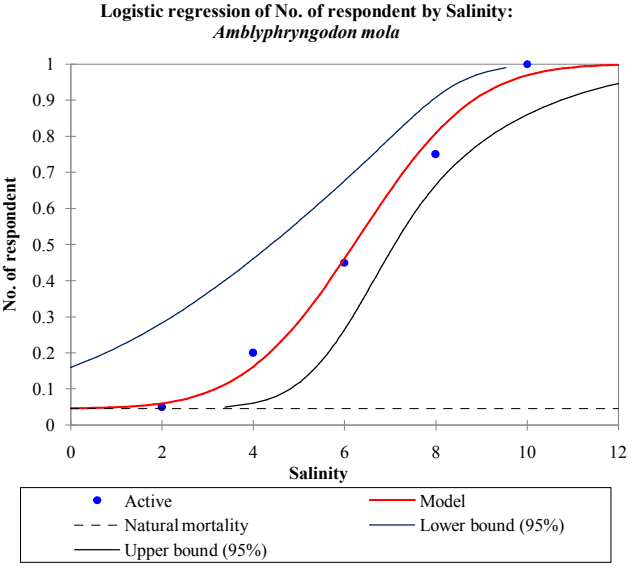

Figure 3: Sigmoid cumulative distribution dose response curve for *Amblypharyngodon mola*.

Lethal Concentration (%)	Salinity concentration (ppt)	Confidence Interval (95%)	
		Lower bound	Upper bound
20	4.35	0.76	5.64
30	5.09	2.21	6.18
40	5.68	3.37	6.63
50	6.20	4.38	7.09
60	6.73	5.32	7.60
70	7.29	6.20	8.24
80	7.93	7.03	9.19
90	8.82	7.92	10.74
95	9.55	8.52	12.15
99	10.92	9.53	14.80

Table 4: Median Lethal Salinity (MLS_{96h}) of *Amblypharyngodon mola* after 96 hour exposure test estimated using probit.

Probability (%)	Salinity concentration (ppt)	Confidence Interval (95%)	
		Lower bound	Upper bound
20	4.27	0.55	5.70
30	5.00	1.16	6.20
40	5.59	2.50	6.64
50	6.12	3.67	7.07
60	6.64	4.78	7.54
70	7.19	5.82	8.14
80	7.82	6.82	9.08
90	8.70	7.79	10.73
95	9.42	8.40	12.30
99	10.77	9.39	15.35

Table 5: Median Lethal Salinity (MLS_{96h}) of *Pethia ticto* after 96 hour exposure test estimated using probit.

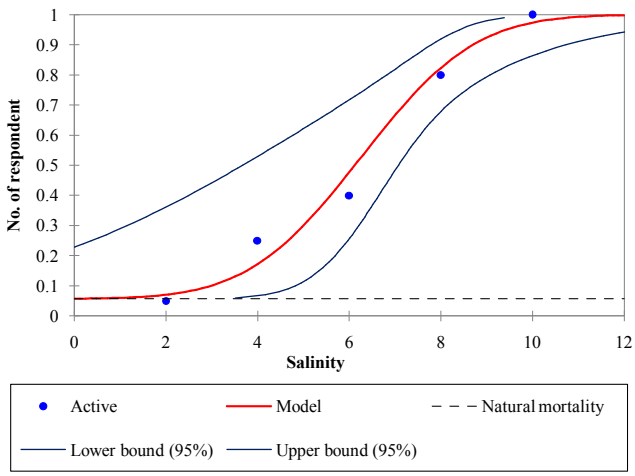

Figure 4: Sigmoid cumulative distribution dose response curve for *Pethia ticto*.

Excessive mucus secretion was observed at moderate level in increasing salinity. Throughout the trial period the control fish remained active and appeared as normal. They also responded well on gentle prodding and stick touch.

Discussion

The investigation from the present study indicate that two small indigenous fish species (SIS), *A. mola* and *P. ticto* inhabiting freshwater areas of Indian Sundarban complex can tolerate within a very narrow range of low salinities. However, Kefford et al., [15] argued that laboratory-obtained survival may not predict survival in nature, let alone the maximum salinity that could support a self-sustaining population. In many natural circumstances, changes in salinity that fish experience would be gradual and this would appear to permit some fish species to tolerate higher salinity than by their direct transfer LC50. Direct transfer LC50 of freshwater fish provided a poorer estimate of the Maximum field distribution (MFD) values [36].

The laboratory derived median lethal salinity value of these small indigenous Asian cyprinids, *A. mola* and *P. ticto* was found 6.20 ppt and 6.12 ppt respectively. The salinity tolerance of these fish are likely similar to other members of the cyprinids. In *Pethia* (*Puntius*) *conchonicus* the maximum tolerance ranges roughly within 8.4 ppt [37]. It was reported that *Catla catla* and *Labeo rohita* fry and fingerlings could tolerate 8 ppt salinity without mortality but survival gradually decreased with increase of salinity [28]. Kasim [29] showed that the upper incipient lethal salinity of *Cirrhinus mrigala*, *Labeo fimbriatus* and *Cyprinus carpio* was 3.54, 7.07, and 8.13 respectively. Garcia [38] found that the median lethal salinity ($MLS_{96\ h}$) of 11-day and 18-day old fry for bighead carp *Aristichthys nobilis* was 2.3 ppt and 6.0 ppt respectively, demonstrating that survival in saline water depends on their age at initial exposure to low salinities. Faizul and Christianus [39] noticed that *Barbodes gonionetus* fry can tolerate salinity up to 10 ppt. The upper salinity tolerance limit of the major primary cyprinids fish, *Pseudophoxinus stymphalicus* was 11.0 ppt [40]. Based on available literature, in cyprinids group, highest lethal salinity limits of 15.6 to 17.2 ppt was documented for Algerian barb *Barbus callensis* from Tunisian Lake Ichkeul [41].

Decreasing survival rates with increasing salinity is characteristic of freshwater stenohaline fish, and it has been postulated that this is due to increasing osmotic maintenance requirements at higher salinities [9]. Optimal salinities for survival of freshwater fish appear to vary according to individual species, life stage and seasonal depended cues. The variations may result from differences in experimental design, temperature optima and other environmental factors, age of the fish, genetic stock and genetic differences between distinct populations [15,42].

In this experiment, both the fish did not show any remarkable stressful sign up to 4 ppt indicated that fish remain unaffected physiologically up to 4-5 ppt. however, hyper-activity, jumping, frequent surface bottom movements, erratic swimming noted after exposing in higher salinity indicated that the fish were approaching their tolerance limit [43]. Respiratory distress like increased opercula movement in higher salinity may be due to excessive mucus secretions. The progressive decrease in the opercular frequency led to swimming close to water surface in order to increase oxygen intake [44,45]. Hyperactivity of fish under any chemical stress, particularly at the initial stage is a frequently observed phenomenon [46].

The ecological classification of freshwater fishes based on their ability to cross marine waters was first proposed by Myers [47]. According to him the primary division species like Cyprinidae unable to enter the sea, are thought to have originated in and dispersed through continental fresh water connections, whereas secondary division species, which are tolerant of seawater salinity and are able to cross marine waters, can colonize coastal basins and continental islands. Chervinski [48] has also stated that there are two types of freshwater fish, so-called primary and secondary freshwater fish. The primary freshwater fish which migrate wholly in freshwater such as

Claridae and Cyprinidae are not able to tolerate salinities higher than 9.8 ppt. In continuation to the fact, Bianco and Nordlie [40] postulated that upper salinity tolerance limit of primary division fish is 12-13 ppt in the gradual increase experiments. The upper limit was lower (about 11.0 ppt) in the sudden increase experiments.

Sea level rise and subsequent erosion coupled with frequent extreme weather events leading embankment failure has a serious and emergent problem in Indian Sundarban over the past two decades. As a results many areas are inundated by brackish water and converting freshwater to oligohaline zone. In this changed scenario, these fish have wider potentialities for culture in low saline brackish water in many areas of Sundarban.

Conclusion

The study suggests that small indigenous fish species *A. mola* and *P. ticto* can potentially be used as a candidate species for aquaculture in slight brackish water areas of Sundarban. However, further studies are required to understand the ecosystem based adaptation processes at higher salinity level, various aspects of culture technologies and to work out the strategies to enhance the adaptability of species to above stress.

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