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# The Survival and Growth of Adult and Juvenile Hard Clam *Meretrix meretrix* Exposed to Variable Estuarine Salinities in Aquarium Settings

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#### **ABSTRACT**

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Salinity fluctuations in estuaries can significantly impact marine organisms, leading to economic losses through mass mortalities of commercially important species. The effects of varying estuarine salinities on the survival, daily mortality, and growth of adult and juvenile hard clam Meretrix meretrix under controlled aquarium conditions were investigated. Adults (shell length ~45 mm) and juveniles (shell length ~30 mm) were collected from an 18 ppt estuarine habitat in Chowfaldondi, southeast Bangladesh, and exposed to six salinity levels (10, 15, 20, 25, 30, and 35 ppt) with three replications at ambient temperature. Adults were monitored for 51 days and juveniles for 23 days. Survival was significantly higher (≥80%) at 15-30 ppt for adults and 10-20 ppt for juveniles, with the lowest survival at 35 ppt. Analysis of co-variance showed that salinity had a greater effect on survival than the combined salinity\*size model (p<0.05). While adult clams exhibited no mortality in first 15 days, juvenile mortality (~10%) began on day two across all salinities with a dose-dependent mortality exceeding 50-80% in 25-35 ppt. Adults were more tolerant to high salinities while juveniles to low salinities. Shell length and width did not vary significantly across salinities, likely due to insufficient plankton in the supplied seawater. This study has implications for potential site selection for coastal clam aquaculture along the Bay of Bengal. The sudden changes and combined effects of multi-stressors on bivalves can be assessed for better understanding how near future climate change can impact bivalve fishery and blue economy.

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# INTRODUCTION

Estuaries are one of the most dynamic and productive coastal environments from the ecological standpoint supporting biologically diverse ecosystems and provide habitat for numerous organisms (Parada et al., 2012; Román et al., 2024). The physico-chemical properties, particularly salinity in estuarine habitats are highly variable, primarily influenced by the tidal action and freshwater discharges, however, may also be influenced due to the diverse range of natural and anthropogenic factors (Elliott and Quintino, 2007). The estuarine environment is brackish in nature where salinity ranges from 0.5 to 29 ppt (Hadley and Whetstone, 2007; Parada et al., 2012). The major factors which may reduce the salinity in estuarine environments are large amount of freshwater input due to precipitation, river runoff and tropical storm, melting of ice, and groundwater flow (Hadley and Whetstone, 2007; Román et al., 2024). Sudden fall of salinity may be more frequent in coming days due to climate change effect which will have negative ecological impact on the structure and functioning of diverse estuarine and marine organisms, particularly that have slow movement capacity (Harley et al. 2006; Pourmozaffar et al., 2020). In contrast, salinity may increase due to evaporation, increase drought event due to temperature increase with climate changes (Hadley and Whetstone, 2007). Fluctuations in salinity may have physiological and immunological effect on an estuarine organism and cause huge economic losses through mass mortalities of commercially exploited stocks (Carregosa et al., 2014; Gajbhiye and Khandeparker, 2017; Parada et al., 2012; Pourmozaffar et al., 2020). The maintenance of stocks of estuarine species strongly depends on the ability of the species to cope with environmental stress (Román et al., 2024). The salinity level of the external water environment directly affects the osmotic pressure of aquatic animals and significantly impact the survival, growth and reproduction (Parada et al., 2012; Wang et al., 2017; Wang and Li, 2018). For example, extreme salinity fluctuation may impair the behavior, physiology and reproduction of marine mollusks and may result in heavy mortalities and economic losses (Gajbhiye and Khandeparker, 2017; Verdelhos et al., 2015).

Salinity is one of the most important abiotic factors that contribute to the distribution of estuarine and marine species, particularly bivalves that cannot move rapidly to avoid stressful environment (Dame and Kenneth, 2011). Salinity affects biological activities, including those related to immune responses, fertilization, development of embryos, survival and growth of larvae and juvenile of marine bivalves (Carregosa et al., 2014; Wang and Li, 2018). The reaction and tolerance of bivalves to changes in salinity levels have been previously evaluated by several investigators (Mcfarland et al., 2013; Cao et al., 2015; Rybovich et al., 2016). As an osmoconformers, bivalves are able to regulate their extracellular and intracellular haemolytic fluids with salinity fluctuations, and they require maintaining a relatively constant fluid concentration for functioning of metabolic enzymes (Baker et al., 2007; Mcfarland et al., 2013; Sokolov and Sokolova 2019). Within the optimal physiological range of salinity, the biological activities are at their maximum (Cao et al., 2015). Salinity variation to optimum range alters filtration rate, heart rate, defense mechanisms, oxygen uptake, survival, growth, and algal consumption of bivalves (Kinne, 1970; Pourmozaffar et al., 2020; Rato et al., 2022; Yuan et al., 2016). It is also evident that the adults and juveniles or the adults of different size classes exhibit varied range of salinity tolerance; i.e. wedge clam Donax trunculus (Reyes-Martínez et al., 2020), mussel Mytella charruana (Yuan et al., 2016), giant clam Tridacna maxima (Mohammed et al., 2019). Understanding the salinity tolerance limit of a commercial estuarine species is the fundamental biological information required to know the ecology, assess the feasibility of large-scale aquaculture, manage overfished bivalve (Manzi et al., 1989; Parada et al., 2012).

Asiatic hard clam genus Meretrix (Veneridae) is an important commercial bivalve in the intertidal and subtidal coastal areas buried in the sand of the seafloor or riverbeds of South and Southeast Asia, including China, Korea, Japan and India and Saudi Arabian Gulf (Admodisastro et al., 2021; Bhattacharya and Sarkar, 2003; Harley et al., 2006; Jayabal and Kalyani, 1986; Wang et al., 2006). Clam play a vital role in coastal ecosystems through filtering phytoplankton from the ocean, regulating water clarity, cycling nutrients, carbon sequestration and making areas more resistant to harmful algal blooms (Bhattacharya and Sarkar, 2003; Chowdhury et al., 2019; FAO 2022). In Bangladesh, the clam, Meretrix sp. is a source of protein and livelihood for coastal people. Indiscriminate overexploitation of clam Meretrix has been noticed around the world, which urges the development of aquaculture to reduce the fishing pressure on natural stock (Admodisastro et al., 2021; Bhattacharya and Sarkar, 2003). Globally, the most of the mollusk production (73%) comes from aquaculture (FAO, 2022). However, clam aquaculture and hatchery development has not developed yet in Bangladesh. Therefore, the understanding the ecological and biological impact of changes in estuarine salinity on hard clam is prerequisite for sustainable coastal bivalve fishery management and aquaculture development. We evaluated the effect of the fluctuating estuarine salinity regimes on the survival and growth of juvenile and adult hard clam Meretrix meretrix in aquarium settings with a view to investigating suitable salinity range for clam aquaculture in the coastal area of Bangladesh. The daily mortality of adults and juveniles in the aquarium settings were also observed. It was also checked whether clam in different salinities can survive with natural sea water supply in the laboratory.

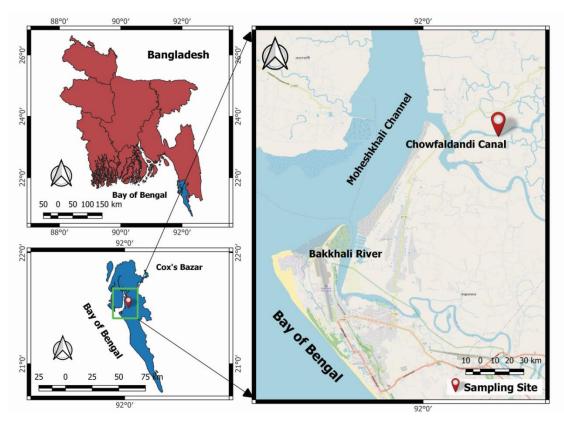
#### MATERIALS AND METHODS

#### Sample collection, study location and duration

The adults and juveniles of hard clam *Meretrix meretrix* were collected from natural habitat, Chowfaldondi canal (21° 30' 40.57119" N and 92° 1' 27.67585" E), Cox's Bazar, Bangladesh during low tide through hiring the traditional tribal clam collectors (Figure 1). Immediately after catch, adult clams were transported to Marine Hatchery of the Coastal Biodiversity, Marine Fisheries, and Wildlife Research Institute (CBMFRI), Chattogram Veterinary and Animal Sciences University in plastic containers filled with the saline water of the collection site (18 ppt). Adult clams were exposed to six salinity treatments (10–35 ppt) for 51 days. Juvenile clams were collected 26 days after stocking of adult clam from the nature (Figure 1), conditioned for two days, and exposed to six salinity treatments for 23 days in the aquariums where adults were stocked.

# **Experimental design**

Our previous study and literature suggest that a large group tribal hard clam collector live in Chowfaldondi, Cox's Bazar and collect clams from the nearby canal called Chowfaldondi Canal. There is no previous study on the seasonal salinity differences in this estuarine canal. However, the salinity in the Bakkhali river estuary (Figure 1), which is just ~5 km away from the collection site and with similar land pattern, varies seasonally from ~12 ppt in monsoon to ~34 ppt post-monsoon (Jahan et al., 2024). Thus, we have selected six salinity treatments starting from 10 to 35 ppt with 05 ppt intervals as 10, 15, 20, 25, 30 and 35 ppt (Figure 2). According to Hadley and Whetstone (2007), hard clams usually reach sexual maturity at a shell length (SL) of about 35 mm (about 1.4 inch). Juvenile clams less than 35 mm SL (about 1.4 inches) is called seed. Jasmin (2017) mentioned that the size at first maturity of the hard clam *Meretrix meretrix* is 40 mm. Therefore, we have selected two size classes: adults (~45 mm SL) and juveniles (~30 mm SL) for our experiment based on abundance in nature.

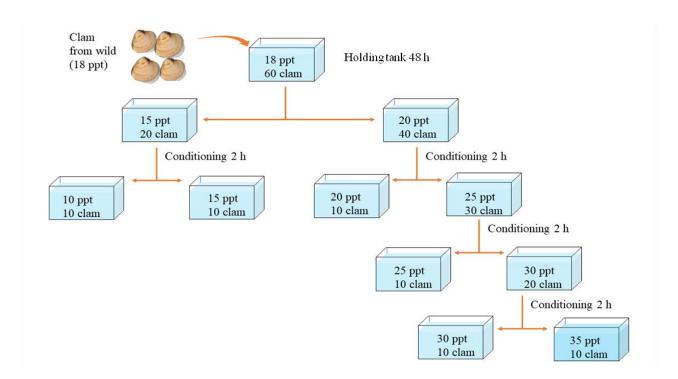


**Figure 1.** Map showing the collection site of hard clam *Meretrix Meretrix* from Chowfaldondi canal, Cox's Bazar district along the southeast coastal area of Bangladesh adjacent to the Bay of Bengal.

# **Acclimatization of clam**

Acclimatization was done to make the collected clams adaptive to the salinity and temperature condition of the experimental units. Firstly, 180 clams were introduced into a three holding tanks with 60 clams in each with 18 ppt water, similar salinity to calm natural habitat. Clams were conditioned for 2 days with continuous aeration and natural sea water supply. Then, from each holding tank (60 clams, 18 ppt), 40 individuals were transferred to 20 ppt tanks and the rest 20 individuals were transferred into 15 ppt tank (Figure 2). After two hours of conditioning, from 15 ppt tank, 10 clams were transferred to 10 ppt tank, and 10 clams remained at 15 ppt tank. Similarly, from 20 ppt aquarium (40 individuals), 30 clams were transferred to 25 ppt tank from which, after two hours of conditioning, 20 clams were transferred to 30 ppt tank. In the same way, after another two hours conditioning, 10 clams were transferred from 30 ppt aquarium to 35 ppt (Figure 2). Thus, finally there were 10 clams in six salinity levels. This process was followed to avoid stress and mortality due to sudden/abrupt change of salinity. Three replications were maintained in 18 aquariums.

After 28 days of adult clam stocking, 270 juvenile clams (~33 mm shell length) were collected from same habitat, and stocked in the same aquariums where adults were stocked. Juveniles were conditioned similar to adults and were separated from the adults using a mesh net.



**Figure 2.** Experimental design showing the process of conditioning and stocking of adult and juvenile hard clam, *Meretrix meretrix* in six salinity treatments (10, 15, 20, 25, 30, and 35 ppt) with three replications of each.

# Aquarium setup

The glass aquariums were washed with detergent, rinsed in freshwater repeatedly and sun-dried for 1 hour. Prior to starting all experiments, salinity treatments were randomly assigned to each tank. Each tank was filled with 16 L of natural seawater water. Air blower was set up to provide aeration to the tanks. Air stones were connected to the tip of rubber tubes and the tubes were then connected to the socket of the air blower. The aeration speed was set to the minimum to prevent damage to clams.

#### Saline water preparation

Natural seawater (30 ppt) was collected from Himchori beach, southeast coast of Bangladesh, is a part of the northern Bay of Bengal through diesel engine pump during high tide. Collected seawater was transported to the hatchery facility of the CBMFWLI, Cox's Bazar. and kept in 1000 L water holding tank to settle the sands and dirt. Then the seawater of 30 ppt salinity was collected from the surface of the tank to ensure the presence of plankton community. Market unrefined salt was added to prepare 35 ppt. Natural seawater was diluted to prepare 10, 15, 20, 25 ppt gradually using formula:

S1 x V1= S2 x V2

Where, S1 = Initial salinity (ppt); V1 = Initial volume (L); S2 = Final salinity (ppt)

V2 = Final volume (L); Freshwater required (L) = V2 - V1

#### Water quality measurement

Salinity (ppt), pH, and temperature (°C) of the aquarium water were measured through digital salinity tester (Hanna – HI98319), digital pH cum thermometer (Hanna – HI98107). As continuous aeration was provided, dissolved oxygen was not measured.

#### Regular maintenance

The values of pH, temperature and salinity in treatment tanks were measured daily and recorded in an entry-book. The salinity of tank water was checked daily and distilled water was added to reduce the salinity that increased due to evaporation. Every three days interval, 50% of the water of rearing tanks was exchanged to reestablish the initial salinity of 10–35 ppt. The experimental tanks were regularly siphoned to remove fecal matter, and any other suspended particles. A rubber tube of 0.5 cm diameter was used for siphoning without disturbing the clams. Besides the cleaning of aggregation of algal population complete removal of dirt and filth of clams was performed through 100% water exchange in every 07 days.

#### Mortality and survival observation

The number of live clams was counted daily during the study period. The dead clams were removed immediately after notice to avoid contamination and reduce stress on clam. Clams with a permanent wide valve gape with extended siphons and a foot that was not responsive within a 5s to touch were considered dead following Carvalho et al. (2015). The mortality data were recorded regularly in a logbook. At the end of the experiment, the average survival (%) in each tank was calculated and compared with the standard of Saurabh et al. (2006) categorizing >80% survival as excellent, 50-80% as fair and <50% as poor. Survival (%) = (Number of clams at the end of experiment/ Initial stocking number of clams) ×100.

#### Plankton observation:

As natural sea water was used in the aquariums for clam rearing, planktons which are available in the aquarium water was identified. Aquarium water was passed through 45 µm plankton net and collected water was preserved with 10% formalin to observe under a compound microscope (Optica, Italy) connected to a digital camera (CB 10) using Sedgwick-Rafter (S-R) counting cell.

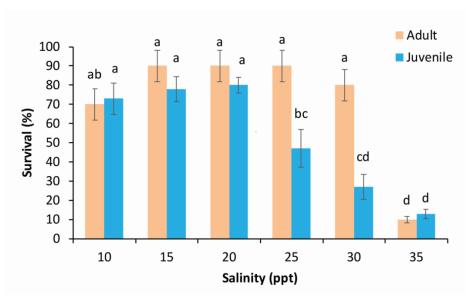
# Statistical analysis

The survival and growth parameters (shell length, shell height) of adult and juvenile clams in six salinity treatments were compared through a one-way analysis (ANOVA) of variance followed by a Tukey's post-hoc test to find the significant differences. The effect of salinity and clam size was determined through One-way Analysis of Covariance (ANCOVA) with considering salinity as a fixed factor and the size of clams as the covariate. In this test, an interaction model (salinity treatments×clam sizes) was also used. In all cases, the Shapiro-Wilk test and Levene's test were conducted to determine the normality and homogeneity of the variance. Data were analyzed through IBM SPSS 25.0 and the level of significance was assigned as 0.05%.

#### **RESULTS**

# Survival of adult and juvenile Meretrix meretrix

In case of adult clams, after 51 days of aquarium observation, significantly higher survival (90%) was found at 15, 20 and 25 ppt (p<0.05). For 10 and 30 ppt treatment tanks, survival was 70% and 80%, respectively. For adults, significantly lower survival (10%) was seen in 35 ppt aquariums. In case of juvenile clams, greater survival was observed at 10, 15, 20 ppt with no significant difference among those treatments. Significantly lower survival 47, 27 and 13% was recorded from 25, 30 and 35 ppt aquariums, respectively with a dose-dependent manner (Figure 3).



**Figure 3.** Survival of adult and juvenile hard clams *Meretrix meretrix* in six salinity exposures in the laboratory condition. Superscripts indicate significant differences assigned within group (adult or juvenile) analyzed through ANOVA and Tukey's post-hoc test.

Analysis of co-variance (ANCOVA) showed that the survival of juvenile and adult clams differed significantly (ANCOVA, F (11,3) = 35.926, p<0.05) (Table 2). Both the salinity treatments (F(5, 35) = 19.583, p<0.05) and clam size (F(1, 35) = 24.347, p<0.05) had significant influence on the survival of clams (Table 2). The interaction between salinity treatments and sizes model suggest that significant (F(5, 35) = 10.48, p<0.05) and moderate influence (Partial Eta Squared = 0.686) on the survival of clams (Table 3). Overall, treatments had a larger effect (Partial Eta Squared = 0.803) on the survival of clam than the size.

Table 1. ANCOVA testing the effects of salinity and size on the survival of clam Meretrix meretrix

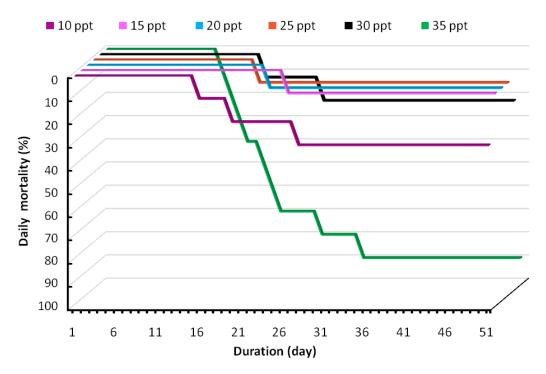
Source	Type III Sum of	df	Mean Square	F	Sig.	Partial Eta
	Squares					Squared
Corrected Model	26687.509a	11	2426.137	35.926	<0.05	0.943
Intercept	26121.98	1	26121.98	386.812	< 0.05	0.942
Salinity	6612.202	5	1322.44	19.583	< 0.05	0.803
Size	1644.167	1	1644.167	24.347	< 0.05	0.504
Salinity × Size	3538.587	5	707.717	10.48	< 0.05	0.686
Error	1620.756	24	67.532			
Total	179980.9	36				
Corrected Total	28308.27	35				

<sup>&</sup>lt;sup>a</sup>R Squared = .943 (Adjusted R Squared = .917)

<sup>&</sup>lt;sup>b</sup>Computed using alpha = .05

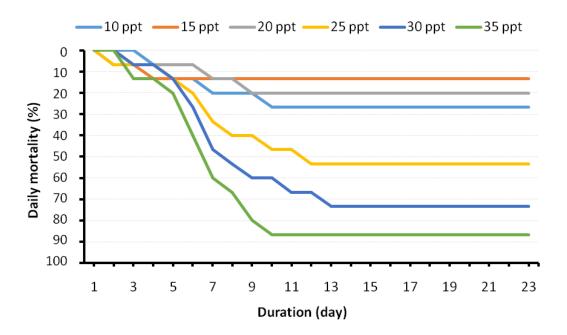
#### Daily average mortality

The adult clams did not experience any mortality within first 15 days in a salinity range 10–35 ppt. After day 15, clams exhibit sudden mortality at higher salinities particularly at 30 ppt and the highest mortality at 35 ppt (Figure 4). However, at 30 ppt 40% adult clams died with day 28 after which mortality was not observed. The survived adults became adapted to that salinity until 51 days of experiment. In case of 35 ppt treatments, the average mortality sharply increased from day 15 to day 36, and only 10% adults survived until the end of the experiment. The mortality in other treatments (10, 15, 20, and 25 ppt) started after day 20 and the total mortality were ~20% at the end of the experiment (Figure 4).



**Figure 4.** A 3-D graph showing the average daily mortality (%) of Adult hard clam *Meretrix meretrix* in six salinity treatments in 51 days observation in the aquarium (10 individuals per treatment with three replications).

In case of juvenile clams, the pattern of mortality was different from adults. The mortality started from the 2<sup>nd</sup> day and >10% mortality was seen for every treatment (Figure 5). Within first 13 days the clam juveniles showed 50%, 70% and 80% mortality in the treatments 25, 30 and 35 ppt, respectively. However, after day 13, no death was seen until day 23 in those treatments. In treatments 10, 15, and 20 ppt, no clam mortality was observed after day 10. The highest percentage of mortality occurred in case of 35 ppt tank. At the end of 23 days the rearing, the observed mortality was the highest (90%) in 35 ppt. Dose-dependent mortality was seen at 25, 30 and 35 ppt salinity with greater 53%, 70% and 87% mortality, respectively. Relatively similar mortality pattern was observed at 10, 15 and 20 ppt salinities (Figure 5).



**Figure 5.** Average daily mortality (%) of juvenile clam *Meretrix meretrix* over 23 days in six salinity treatments (15 individuals per treatment with three replications).

# Plankton observation

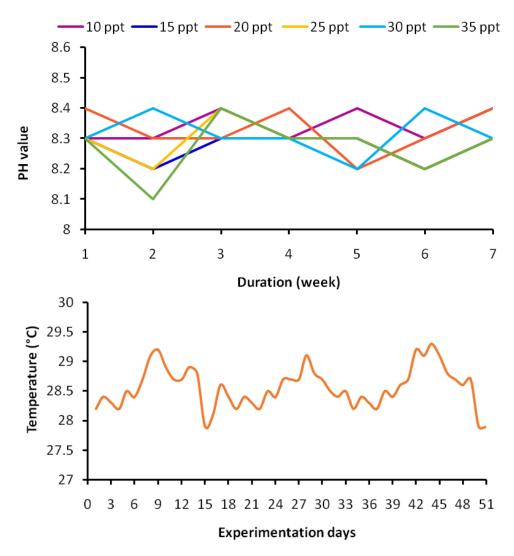
Aquarium water which is directly collected from sea, contain some types of planktons which are observed under microscope. For instance, *Cyclotella* sp., *Pleurosigma* sp., *Navicula* sp. under Bacillariophyceae and Cladocerans zooplankton were observed commonly in natural sea water.

#### Water quality of the rearing tanks

The mean value of pH varied from  $8.25\pm0.01$  to  $8.35\pm0.05$  in all the aquarium. Actual value of pH of the treatment tanks change from 8.1 to 8.4 which was taken weekly (Figure 6). Environment temperature ranged from  $27.9^{\circ}$ C to  $29.4^{\circ}$ C and the average temperature was  $28.56\pm0.34^{\circ}$ C (Figure 6).

#### Size variation

For adult clams, the initial average shell length and shell width was 44.30±0.97 and 24.84±1.08, and the average final shell length and shell width was 44.46±0.97, 24.89±0.98, respectively. The growth parameters did not vary significantly among the salinity treatments (Table 3). In small clams, the initial and final average shell length was 30.68±2.45 and 30.71±2.50, respectively with no significant differences in growth parameters among salinity exposures. The initial average shell width was 16.30±1.54 while final average shell width was 16.33±1.53 (Table 4).



**Figure 6.** Average pH value for six salinity exposure treatments and temperature record during clam rearing for 51 days at Cox's Bazar, Bangladesh.

**Table 3.** Variation in the growth parameters of adult clam *Meretrix meretrix* over 51 day of aquarium rearing among six salinity treatments using ANOVA.

Growth parameters	Salinity treatments (ppt)						
	10	15	20	25	30	35	-
Initial shell length	44.40±0.41	44.25±0.68	44.44±0.49	44.47±0.55	44.47±0.53	44.14±0.31	0.95
	(44.10-44.87)	(43.46-44.64)	(44.10-45.01)	(44.10-45.10)	(44.10-45.08)	(42.80-44.40)	
Final shell length	44.54±0.41	44.41±0.68	44.59±0.50	44.63±0.54	44.63±0.54	44.30±0.31	0.95
	(44.26-45.01)	(43.62-44.80)	(44.26-45.17)	(44.26-45.25)	(44.26-45.25)	(43.95-44.56)	
Shell length	0.14±0.01	0.16±0	0.15±0.02	0.15±0.005	0.16±0.01	0.16±0.005	0.49
increment	(0.13-0.16)	(0.16-0.16)	(0.12-0.16)	(0.15-0.16)	(0.15-0.17)	(0.15-0.16)	
Initial shell width	24.47±0.15	24.33±0.10	24.65±0.26	24.38±0.74	24.99±0.63	24.38±0.24	0.45
	(24.38-24.65)	24.21-24.39	(24.38-24.91)	(23.54-24.95)	(24.38-25.64)	(24.21-24.65)	
Final shell width	24.52±0.15	24.39±0.10	24.70±0.27	24.44±0.75	25.05±0.63	24.43±0.23	0.45
	(24.43-24.70)	(24.27-24.45)	(24.43-24.96)	(23.59-25.02)	(24.43-25.69)	(24.27-24.70)	
Shell width	0.05±0	0.06±0.005	0.05±0	0.06±0.01	0.06±0.01	0.05±0.006	0.71
increament	(0.05-0.05)	(0.05-0.06)	(0.05-0.05)	(0.05-0.07)	(0.05-0.07)	(0.05-0.06)	

**Table 4.** Variation in the growth parameters of juvenile clam *Meretrix meretrix* over 23 days of aquarium rearing in six salinity treatments using ANOVA.

Growth parameters	Salinity treatments (ppt)							
	10	15	20	25	30	35	=	
Initial shell	30.98±2.82	30.02±0.57	30.38±1.98	30.53±0.43	30.09±1.92	30.46±2.96	0.99	
length	(28.17-33.81)	(29.41-30.54)	(28.17-32.01)	(30.1-30.96)	(28.17-32.01)	(28.17-33.81)		
Final shell	31.07±2.82	30.10±0.57	30.46±1.98	30.62±0.41	30.18±1.92	30.55±2.96	0.99	
length	(28.26-33.9)	(29.49-30.63)	(28.26-32.08)	(30.19-31)	(28.26-32.1)	(28.26-33.9)		
Shell length	0.09±0.00	0.09±0.005	0.08±0.01	0.08±0.04	0.09±0.00	0.09±0.00	0.99	
increament	(0.09-0.09)	(0.08-0.09)	(0.07-0.09)	(0.04-0.12)	(0.09-0.09)	(0.09-0.09)		
Initial shell	16.39±0.63	16.03±0.42	16.56±1.01	16.12±0.89	16.24±0.85	16.03±0.42	0.93	
width	(15.86-17.09)	(15.72-16.51)	(15.39-17.2)	(15.39-17.11)	(15.39-17.09)	(15.72-16.51)		
Final shell	16.43±0.62	16.06±0.42	16.59±1.02	16.15±0.90	16.27±0.84	16.06±0.43	0.93	
width	(15.89-17.11)	(15.75-16.54)	(15.42-17.24)	(15.42-17.16)	(15.42-17.11)	(15.75-16.55)		
Shell width	0.03±0.01	0.03±0	0.03±0.005	0.03±0.02	0.03±0.01	0.03±0.005	0.99	
increament	(0.02-0.05)	(0.03-0.03)	(0.03-0.04)	(0.01-0.05)	(0.02-0.04)	(0.03-0.04)		

# DISCUSSION

# Survival of adult and juvenile M. meretrix

The significantly greater survival of adult clams within 10–30 ppt (≥80%) indicating a suitable range of clam survival in laboratory condition. However, for the juveniles, the suitable range was 10–20 ppt. Following Saurabh et al. (2006), range of survival is ranked as excellent. The lowest survival for both adult and juvenile clams were at 35 ppt treatment indicating that though *M. meretrix* is a euryhaline species, the optimum range for survival and potentially for growth would be within 30 ppt. However, the upper range of salinity tolerance was different for adult and juveniles; juveniles survived better at 10–20 ppt and adults could better tolerate higher salinities than the juveniles. The reason behind this may be that juvenile clams are more susceptible to salinity change, agreeing with Baker et al. (2007).

The survival of adults was lower than juveniles at 10 ppt indicating adults are more susceptible to low salinity than juveniles. It is also the case for yellow clam *Mesodesma mactroides*, which is a moderatly euryhaline species, could tolerate 15–35 ppt salinities, where adults were less tolerant to low salinity and suffer severe mortality if rain or flood water continues for several days (Carvalho et al., 2015). Similarly, wedge clam *Donax trunculus* is a euryhaline species in which the tolerance to low salinities varied between two-size classes; salinities below 14.2 ppt were lethal for both size classes, however juveniles could resist low salinities more than the adults (Reyes-Martínez et al., 2020). According to Kinne (1970), the greater tolerance of juveniles to low salinities than adults could be explained by the additional metabolic demand due to the onset of gonad maturation. However, significantly lower survival of *Meretrix* was found at 35 ppt in our study.

The tolerance to salinity ranges are species specific and will not be the same for all species (Parade et al., 2012). For example, Baker et al. (2007) suggested that the optimum range of salinity for hard clam *Mercenariamercenaria* is 20–30 ppt. According to Cao et al. (2015), hard clam can survive at (9 to 39.5) ppt salinity at natural habitat, when the salinity exceeded 40 ppt, the survival rate of the individuals decreases significantly. Some bivalves are more tolerant to salinity variations. For example, ark shell *Anadara broughtonii* was exposed to salinities 15, 18, 21, 24, 27 and 30 PSU under hatchery for a period of 25 days showed more than 98% survival in all treatment groups (Wang et al., 2017). Iwagaki oyster *Crassostrea nippona* showed the maximum survival rate of 84.44% at salinity 20 ppt with no significant variation among the six salinities (15, 20, 25, 30, 35, and 40) treatments (Wang and Li, 2018). Survival of *Donax trunculus* was ~100% for both adult and juveniles from 26.6 to 36.7 ppt (Reyes-Martínez et al., 2020). Chowdhury et al. (2019) mentioned that *M. meretrix* was found between 12.36 to 26 ppt from the Moheshkhali Island, Bangladesh, which also supports that survival of clam may be lower above 30 ppt and below 10 ppt. However, we did not include salinities below 10, which could also provide evidence of lower survival in low salinity.

#### **Mortality**

Our study suggests that both adult and juvenile *M. meretrix* could endure a wide range of salinity 10–35 ppt similar to euryhaline hard clam *Mercenaria mercenaria* (Baker et al., 2007). In this experiment, adult *M. meretrix* did not experience mortality in first 15 days indicating a wide range of salinity tolerance capacity of this species. However, rapid mortality started around day 15 in both 30 and 35 ppt experienced more than 60 and 90% mortality at the end of 51-day laboratory observation. In case of juveniles, mortality started from 2<sup>nd</sup> day though 50% mortality reached only after day 6. Juveniles experienced 53, 73 and 87% mortality at 25, 30 and 35 ppt. Therefore, the determination of 50% mortality (Lt50) for adult is not possible, and for juveniles it requires more than 5 days. Similarly, the Lt<sub>50</sub> determination for both juveniles and adults of european clam *Ruditapes decussatus* required higher than 120 h (Rato et al., 2022). Baker et al. (2007) found that increased mortality in *M. mercenaria* clams began at around day 13 when exposed to 5 ppt, while mortality started early from day 04 at 45 ppt.

The mortality pattern in *M. meretrix* supports the idea that although estuarine hard clams can tolerate a wide range of salinities, they cannot tolerate sudden and very low or high salinity for prolonged period (Baker et al., 2007; Shumway et al., 1977). However, very few adults and juveniles could tolerate 35 ppt through the experimental period suggesting that after a period of salinity adaptation, bivalves can endure beyond their specific range of salinity (Cao et al., 2015). They observed that the hard clams died at salinity >30 ppt, however, when salinity is below 40 ppt, some of the animals began to adapt to the water environment and the mortality decreased as time goes on. Castagna and Chanley (1973) examining 36 species of bivalves also suggested that small incremental changes in salinity may allow species to broaden their tolerance threshold. Sediment burial is one of the major mechanisms used by clams to isolate themselves from stress (Shumway et al., 1977). However, in this study, the hard clams were kept in aquariums devoid of sand. If the clams were allowed to dig a burrow in the sand, they might have had a longer lifespan which need to be tested.

# Growth of adult and juvenile M. meretrix

The growth parameters (shell length and shell width) of adult and juvenile *M. meretrix* observed for 51 and 23 days, respectively did not show significant variation salinity-wise. The increment in shell length and width was minimal in the aquarium system potentially due to the absence of sufficient phytoplankton in the supplied natural seawater. The presence of phytoplankton genus *Cyclotella*, *Pleurosigma*, *Navicula* in the natural seawater used in rearing aquariums supports that diatom is the dominant group of phytoplankton in Bay of Bengal throughout the year (Jewel et al., 2002). Clams will basically eat anything that's small enough to go through their siphons, and that makes them low maintenance even when in captivity. They feed on planktons, bacteria, microorganisms, and even fish excrement (Dame and Kenneth, 2011). Slightly higher growth rate in juvenile clam that were reared for 23 days than the adults that were reared for 51 days supports Mohammed et al. (2019) findings that the growth rate of juvenile giant clams (*Tridacna maxima*) was significantly higher than the adults and decreased with increasing clam size.

In the appropriate range of salinity, animals can survive and grow optimally. Within the optimum range, clam shows the maximum pumping and feeding rates, growth (Baker et al., 2007). When the salinity exceeded the appropriate salinity range, it will affect the survival and normal growth of aquatic animals. In response to low salinity stress caused by strong rainfalls, the native clams *Ruditapes decussatus* and *Venerupis corrugata* demonstrated significant decreases in oxygen consumption, clearance rate, and growth (Mcfarland et al., 2013; Román et al., 2024). Ark shell *A. broughtonii* were exposed to salinities 15, 18, 21, 24, 27 and 30 PSU for a period of 25 days showed the growth rate was higher in higher (30 PSU) salinities (Wang et al., 2017). Similarly, Iwagaki oysters *Crassostrea nippona* examined in 15–40 ppt salinities, showed the highest mean shell height and growth rate at the salinities 25 and 30 (Wang and Li, 2018). However, we did not observe this growth variation in salinity exposures potentially due to insufficient food supply.

The accepted pH level in a basic saltwater system is between 7.6 and 8.4, but indoor tanks are more sensitive, and therefore need to be kept at the higher end of the pH scale, 8.0 to 8.4 (Arnold et al., 2000). Treatment tanks had mean pH value ranges from 8.1 to 8.4, alike seawater and within the suitable range following Dame and Kenneth (2011) and Arnold et al. (2000). The temperature value of this study tanks falls within the suitable range for hard clam suggested by Yuan et al. (2016) 20–30°C, and Hadley and Whetstone (2007) 27°C to 29°C.

In this study, we observed the effect of salinity, a single parameter, on survival and growth of clam. However, in nature, there are multiple factors among which temperature is one of the most important factors that influence the survival and distribution of a species. For instance, mussel *Mytella charruana* juvenile and adult individuals can survive in a wide range of salinities (5–40 ppt) at 20°C, but their salinity tolerance range narrowed as the temperature decreased or increased (Yuan et al., 2016). Similarly, *Perna viridis* can survive at a wide range of temperatures (9–35°C) when the salinity is 35–37 ppt; however, as salinity decreased, the thermal survival ranges for P. *viridis* became narrower (Yuan et al., 2016). European clam *Ruditapes* 

decussatus juveniles and adults showed mortality <15 ppt indicated that high heavy precipitation due to climate change would impede the recruitment (Rato et al., 2022). Both juveniles and adults *R. decussatus* experience higher and faster fatalities in low salinities and high temperatures (Rato et al., 2022). Therefore, future endeavors on understanding the effect of multi stressors on the biology and physiology of bivalves akin to nature are required.

#### CONCLUSION

The Asiatic hard clam *M. meretrix* demonstrates considerable adaptability to varying salinity levels from 10 to 35 ppt. Adults were more resilient to high salinities while juveniles show greater resilience at lower salinities. These findings underscore the importance of considering salinity preferences in the site selection process for commercial aquaculture along Bangladesh's northern Bay of Bengal coast. For sustainable bivalve culture, a deeper understanding of how combined environmental stressors affect the behavior, physiology, and ecology of *M. meretrix* is essential.

# **COMPETING INTEREST**

The authors declare that they have no competing interests

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