



Effect of Salinity Regimes on Growth Performance, Digestive Enzyme Activity and Histological Structure of *Scylla serrata*

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ABSTRACT

Background: *Scylla serrata* is a high-value aquaculture species is widely farmed in Asia due to its adaptability and market demand. Among crucial environmental parameters, salinity has a significant impact on development, osmoregulation, digestive efficiency and overall growth performance. Although the larval and juvenile phases have been the main focus of earlier research, the effects of salinity under controlled conditions have also been examined. Thus, standardizing the ideal salinity for the grow-out stage is the objective of this study.

Methods: A controlled eight-week experiment was carried out to assess the effects of three salinity regimes (25, 30 and 35 ppt) on the growth performance, digestive enzyme activity and tissue histology of *S. serrata*. Mud crabs (400.00±10.00 g) were stocked in triplicate groups and fed a natural diet.

Result: The study observed better growth performance and survival in 30 ppt salinity group. The significantly highest weight gain (74.51±4.78 g), weight gain % (18.13±1.29%) and average daily growth (1.24±0.08 g/day) were observed in 30 ppt compared to 25 and 35 ppt treatments. Digestive enzyme activities also showed significant differences ($p<0.05$) among treatments in both hepatopancreas and midgut tissues, with the highest activities observed in the 30 ppt (T2). Histologically, midgut structure remained unchanged by treatments; however, hepatopancreas tissues at 35 ppt revealed cellular abnormalities, including sloughed microvillar structures and restricted tubular lumens, indicating salt-induced stress. Water quality measurements remained within optimum ranges, while hardness and alkalinity changed dramatically with salt levels. A salinity level of 30 ppt enhances ideal growth, feed utilization and physiological integrity in *S. serrata* while minimizing histological damage. These findings highlight the importance of salinity management in mud crab aquaculture and support the selection of 30 ppt as the best practice salinity regime for sustainable and efficient production.

Key words: Digestive enzymes, Growth performance, Hepatopancreas histology, Mud crab, Salinity.

INTRODUCTION

Aquaculture is one of the fastest-growing food production sectors, providing a high-protein and sustainable alternative to wild-caught aquatic organisms. With rising seafood demand and declining capture fisheries, many nations are actively developing cultivation technologies for new species (Zhao *et al.*, 2025). Among these, mud crabs have emerged as a globally important aquaculture commodity, with increasing demand in both domestic and international markets.

Edible crabs are predominantly distributed in marine and brackish water environments, with most commercially important species belonging to the family Portunidae (Josileen, 2023). Within this family, *Scylla* spp., commonly known as mud crabs, are widely farmed across Asia due to their rapid growth, high market value and adaptability to aquaculture systems (Nguyen *et al.*, 2022). These crabs typically inhabit mangrove wetlands and estuarine mudflats influenced by tidal fluctuations. Their farming is considered highly lucrative, supported by several advantages such as simple culture techniques, reuse of disused shrimp ponds, well-established global markets, natural abundance across tropical Asia, ease of handling and transport, adaptability to both small-and large-scale farming and higher market prices compared to penaeid shrimps (Zhao *et al.*, 2025).

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Since the 1980s, *Scylla serrata* has been recognized as the most commercially significant species due to its fast growth, high consumer demand and strong export potential across Southeast Asia and beyond (Syafaat *et al.*, 2021). Successful cultivation of *S. serrata* depends on multiple environmental parameters, among which temperature and salinity play a pivotal role in regulating feed efficiency, growth rate, survival and overall health (Soundarapandian *et al.*, 2009). Salinity, in particular, is a critical factor influencing osmoregulation, nutrient assimilation, metabolic performance and survival in mud crabs (Minagawa, 1992). Deviations from the optimal salinity range can impose physiological stress, diverting energy away from growth and metabolism toward osmoregulatory processes. This leads to reduced feed intake, impaired digestion, higher disease susceptibility and mortality (Wang *et al.*, 2022). The effects are especially pronounced during juvenile and grow-out stages, when nutrient requirements are high to support rapid biomass accumulation (Long *et al.*, 2023).

Although, several studies have investigated the influence of salinity on larval development and seed production of *S. serrata* (Millamena *et al.*, 2001), research on the grow-out phase under controlled salinity regimes remains limited. Ruscoe *et al.* (2004) reported that juveniles from Northern Australia achieved optimal growth and survival at salinities of 10-25 ppt. Similarly, Mia and Shah (2010) demonstrated that survival and growth of crab larvae significantly improved when salinity increased from 5 to 25 ppt. More recently, Eddiwan *et al.* (2021) showed that juvenile crabs grew best at 27-33 ppt, with maximal growth occurring at salinity reduced by 4 ppt from full seawater (35 ppt) without compromising survival.

Despite these findings, comprehensive evaluations of salinity effects on growth performance, nutrient digestibility and survival during the adult grow-out stage are scarce. Addressing this knowledge gap, the present study investigates the influence of different salinity regimes on *S. serrata* under controlled experimental conditions. The outcomes are expected to provide critical insights for evidence-based management strategies in mud crab fattening and hatchery operations, thereby enhancing the sustainability and profitability of mud crab aquaculture.

MATERIALS AND METHODS

Ethical statement

The experiment was conducted following the procedures of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research. The study was approved by ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam (TNJFU, 2024), Tamil Nadu, India.

Experimental design and experimental setup

Healthy and uniform sized 50 individuals of *S. serrata* (average weight of 400.00 ± 10.00 g and average length

126.13 ± 1.02 mm) were procured from Pazhaverkadu fish market (Lat. 13.422832° N, Long. 80.296034° E), Tamil Nadu, India and transferred to the Pulicat Research Field Facility, Pazhaverkadu, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India. The crabs were acclimatized in the FRP circular tanks ($2.0 \text{ m} \times 0.76 \text{ m}$; dia $\times$ height) of 2000 L capacity at a stocking density 4 crabs/2000 L and the required salinity for treatment were adjusted gradually. After the acclimatization, crabs were divided into three treatments in triplicates stocked at 12 crabs/treatment. The treatments for the present study are 25, 30 and 35 ppt. Each group was reared in triplicate for eight weeks and fed a natural diet consisting of fish, shrimp and squid procured from the Pazhaverkadu fish market. Feeding was done daily at 09:00 and 17:00 hours at a 10% of body weight and feeding was adjusted by subsequent sampling. Continuous aeration was provided throughout the experimental period using an air blower.

Water quality parameters

Throughout the experimental period, water quality parameters such as Salinity, Temperature, Dissolved oxygen, pH, Total ammonia-N, nitrite-N, nitrate-N, water hardness and alkalinity were measured. 10% of water has been exchanged daily to maintain optimum water quality parameters in the experimental tanks and salinity was adjusted. The salinity, pH and Dissolved Oxygen of water was measured using the laboratory model combo water testing meter multi parameter water tester AZ 86031, India. Total alkalinity, Ammonia -N, nitrite-N, nitrate-N and Total hardness was determined following the standard methods (APHA, 2005).

Growth parameter and nutrient utilization

At the end of experimental trial, all the crabs were individually weighed to estimate the average weight gain (WG), weight gain percentage (WG %), Average daily gain (ADG), Specific Growth Rate (SGR) and survival % (Geoffery *et al.*, 2024; Jackquelinwino *et al.*, 2025) were calculated as:

$$\text{Weight gain (WG)} = \text{Final wet weight (g)} - \text{Initial wet weight (g)}$$

$$\text{Weight gain (WG \%)} =$$

$$\frac{\text{Final wet weight (g)} - \text{Initial wet weight (g)}}{\text{Initial wet weight (g)}} \times 100$$

$$\text{Average daily gain (ADG)} =$$

$$\frac{\text{Final wet weight (g)} - \text{Initial wet weight (g)}}{\text{Experimental duration}}$$

$$\text{Specific growth rate (SGR)} =$$

$$\frac{\ln \text{Final weight (g)} - \ln \text{Initial weight (g)}}{\text{Experimental duration}} \times 100$$

$$\text{Survival \%} = \frac{\text{Final number of crabs}}{\text{Initial number of crabs stocked}} \times 100$$

Digestive enzyme analysis

At the end of the experimental trial three crabs per treatment were collected and submerged in ice slurry (1:1 ratio of crushed ice to water) for 5 minutes until the crab movement was ceased. Hepatopancreas and midgut were collected for digestive enzymes analysis. A homogenizer was used to mix the collected tissues with 0.25 M sucrose. Later the sample was homogenized at 3,070 g and supernatant was collected. The supernatant was used for further analysis of protease, lipase and amylase enzyme activity. Protease activity was determined using the casein digestion method (Sarath, 1989; Joshna *et al.*, 2024), Lipase activity was estimated by the titrimetric method using a stabilized olive oil emulsion as substrate (Cherry and Crandall, 1932) and Amylase activity was analyzed using dinitro salicylic acid method (Clark, 1964; Priyatharshni *et al.*, 2024). Except for lipase, all enzyme activities were quantified using a spectrophotometer based on absorbance changes and expressed as units per milligram of protein per minute (U/mg protein/min). One unit of protease, lipase and amylase activity corresponded to the release of 1 µg of tyrosine, fatty acid and maltose per minute, respectively.

Histology

At the end of the experimental trial, three crabs per treatment were collected and anesthetized using ice slurry (1:1 ratio of crushed ice to water) for 5 minutes until the crab movement was ceased. Later midgut and hepatopancreas were collected for histopathology studies and stored in 10% neutral buffered formalin. Tissues were initially fixed in neutral buffered formalin (NBF), followed by dehydration through a graded ethanol series. Then they were cleared using xylene, embedded in liquid paraffin wax and blocked at 58°C. The paraffin-embedded tissues were sectioned at 6 µm using a rotary microtome (Leica RM2255, India). Hematoxylin and Eosin staining was carried out using a Microm HMS7. The prepared slides were examined under a microscope (Leica E200, India) at 20X magnification and using for further analysis.

Statistical analysis

All of the data were examined for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test). The present data were represented using mean and standard

deviation (SD) values of the triplicates. One-way ANOVA, followed by Duncan's test at the significant level of 0.05 was used to compare among the treatments. The data were statistically analysed by SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA). Histological analysis of the crab hepatopancreas and midgut were explained descriptively.

RESULTS AND DISCUSSION

Growth performance

At the end of the experiment, the present study found significant difference in growth performance of mud crab reared at different salinity (Table 1). Significantly higher values of weight gain (74.51±4.78 g), weight gain % (18.13±1.29%) and average daily gain (1.24±0.08 g/day) was recorded at 30 ppt salinity treatments. Interestingly, no significant ($P>0.05$) difference was observed in survival rate and specific growth rate in between the experimental groups. Growth parameters are key biological indicators that help assess the suitability of environmental conditions, such as salinity, for the culture of *Scylla serrata* (Eddiwan *et al.*, 2021). Similarly, Eddiwan *et al.* (2021) reported that reducing salinity from 33 ppt to 29 ppt had significantly enhanced the growth performance of juvenile mud crab (*Scylla serrata*) at 29 ppt. Mia and Shah (2010) reported that increasing salinity levels from 5 ppt to 25 ppt significantly enhanced the growth performance of crabs, indicating a positive correlation between salinity and growth under controlled conditions. Contradictory, Suyono (2021) found that mud crabs raised at 20 ppt salinity performed better in terms of weight gain and average daily growth. This study showed improved growth at 30 ppt salinity, suggesting that the therapy created better physiological conditions that supported growth. Potentially as a result of adequate osmoregulatory balance at that salinity level, more effective feed utilization and energy partitioning may be responsible for the notable improvements in weight gain (WG) and average daily gain (ADG). Because maintaining ionic and osmotic equilibrium can result in large energetic costs under suboptimal salinities, salinity is crucial to the metabolic efficiency of euryhaline organisms like *Scylla serrata*. Numerous research have demonstrated that raising mud crabs at salinity levels that lessen the energetic cost of osmoregulation and free up more energy

Table 1: Growth performance of *Scylla serrata* reared at different salinities.

Parameters	25 ppt	30 ppt	35 ppt	P value
Initial weight (g)	411.17±4.93 ^a	411.25±3.36 ^a	411.58±5.69 ^a	0.975
Final weight (g)	476.03±5.60 ^c	485.76±2.88 ^a	479.58±2.46 ^b	0.000
Weight gain (g)	64.87±7.32 ^b	74.51±4.78 ^a	67.99±6.16 ^b	0.002
Weight gain percentage (%)	15.79±1.91 ^b	18.13±1.29 ^a	16.54±1.72 ^b	0.005
Average daily gain (g)	1.08±0.12 ^b	1.24±0.08 ^a	1.13±0.1 ^b	0.002
Specific growth rate (%/day)	0.0017±0.00034 ^a	0.0018±0.00012 ^a	0.0017±0.00016 ^a	0.199
Survival rate (%)	83.75±8.83 ^a	89.17±12.03 ^a	84.58±11.76 ^a	0.437

Note: Values were expressed as mean ± standard deviation. Values in the same row with different superscripts are significantly different at $p<0.05$.

for somatic growth enhances their growth performance (Mia and Shah, 2010; Suyono, 2021). According to the study, a salinity level of 30 ppt is likely ideal for the *S. serrata*, which matches what has been observed. Stable salinity may also help improve food absorption and protein retention, which can support growth.

While there was no significant difference in SGR or survival between treatments, 30 ppt salinity consistently displayed a trend toward greater survival (89.17±12.03%). But there was no statistically significant difference in SGR or survival across the treatments. This pattern suggests that 30 ppt salinity level might have produced a more stable physiological environment, lowering the risks of stress and death. Previous research has shown that appropriate salinity levels enhance better survival outcomes by increasing osmoregulatory efficiency and lowering the energy needed to maintain ionic balance (Ruscoe *et al.*, 2004). The osmoregulation of crabs under stress conditions of salinity resulted in loss of energy for growth (Pourmozaffar *et al.*, 2020). Furthermore, it has been demonstrated that mud crab survival rates peak within a particular salinity range; beyond this, physiological stress rises, which may result in stunted growth or increased death (Baylon *et al.*, 2010). Therefore, although not statistically significant, the consistently higher survival in 30 ppt suggests a salinity level more closely aligned with the species optimal tolerance threshold.

Water quality parameters

Throughout the experiment, all physicochemical parameters remained within optimum and acceptable range, whereas salinity, calcium hardness, magnesium hardness, total hardness and alkalinity exhibited significant differences among treatments (Table 2). Significantly higher values of calcium hardness - 227.13±13.78 mg/l; magnesium hardness - 661.93 ± 39.63 mg/l and alkalinity - 127.87±3.61 mg/l was recorded at 30 ppt salinity treatment. Salinity levels were significantly different, measured at 25.40±0.22 ppt in T1 (25 ppt), 30.42±0.23 ppt in T2 (30 ppt) and 35.30±0.16

ppt in T3 (35 ppt) respectively. The study recorded water quality parameters such as temperature - 28.91±0.24°C; pH - 8.25±0.05; dissolved oxygen - 5.70±0.64 mg/l; ammonia - 0.022±0.009 mg/l; nitrite - 0.024±0.007 mg/l; nitrate - 0.0251±0.008 mg/l, respectively. The growth, survival and effectiveness of crab molting are all significantly impacted by water quality parameters. Better feed conversion and growth performance are encouraged by ideal circumstances, which also lessen stress and boost metabolic processes (Yulianto *et al.*, 2019).

Similarly, Rezaei *et al.* (2015) has also reported that calcium hardness increased dramatically with increasing salinity ($P < 0.001$), most likely as a result of higher ionic concentrations in saline environments. The behavior of divalent ions such as Mg^{2+} under salinity stress was reflected in the comparable pattern of magnesium hardness ($P = 0.035$). In line with findings by Aruna and Felix (2017), total hardness, a measure of calcium and magnesium combined, increased significantly. Additionally, alkalinity increased dramatically ($P = 0.020$) (Lauritzsen *et al.*, 2016). Elevated salinity dramatically changed water hardness and alkalinity, which may affect aquatic animal physiology and should be monitored in aquaculture systems, even when fundamental water quality indicators like temperature, pH and DO stayed constant.

Digestive enzyme analysis

Digestive enzyme activity is a key parameter in evaluating an organism's ability to digest and utilize nutrients effectively. Different salinity treatments had significantly affected the protease, lipase and amylase activities of hepatopancreas and mid gut (Fig 1, 2 and 3). Hepatopancreas of *S. serrata* reared in 30 ppt salinity showed significantly higher levels of protease (0.0070±0.00016 U/mg protein/min), lipase (1.0121±0.0819 U/mg protein/min) and amylase (0.0189±0.00031 U/mg protein/min) activities. On the other side, significantly higher protease (0.0065±0.00033 U/mg protein/min), lipase (0.9575±0.0125 U/mg protein/min) and amylase (0.0198±0.00031 U/mg protein/min)

Table 2: Water quality parameters of *Scylla serrata* reared at different salinities.

Parameters	25 ppt	30 ppt	35 ppt	P value
Salinity	25.40±0.22 ^c	30.42±0.23 ^b	35.30±0.16 ^a	<0.001
Temperature (°C)	28.83±0.16 ^a	28.91±0.24 ^a	28.68±0.16 ^a	0.083
pH	8.23±0.02 ^a	8.25±0.05 ^a	8.21±0.05 ^a	0.374
Dissolved Oxygen (mg/l)	5.70±0.64 ^a	5.70±0.64 ^a	5.37±0.77 ^a	0.372
Ammonia (mg/l)	0.019±0.003 ^a	0.022±0.009 ^a	0.020±0.004 ^a	0.777
Nitrite (mg/l)	0.022±0.005 ^a	0.024±0.007 ^a	0.026±0.004 ^a	0.120
Nitrate (mg/l)	0.0200±0.005 ^a	0.0251±0.008 ^a	0.0236±0.007 ^a	0.195
Calcium hardness (mg/l)	189.73±12.17 ^b	218.73±12.33 ^a	227.13±13.78 ^a	<0.001
Magnesium hardness (mg/l)	568.60±19.85 ^b	626.47±20.80 ^a	661.93±39.63 ^a	0.035
Total hardness (mg/l)	3433.43±121.10 ^c	3741.07±102.06 ^b	4175.40±88.03 ^a	<0.001
Alkalinity (mg/l)	117.80±4.89 ^b	122.93±4.58 ^b	127.87±3.61 ^a	0.020

Note: Values were expressed as mean ± standard deviation. Values in the same row with different superscripts are significantly different at $p < 0.05$.

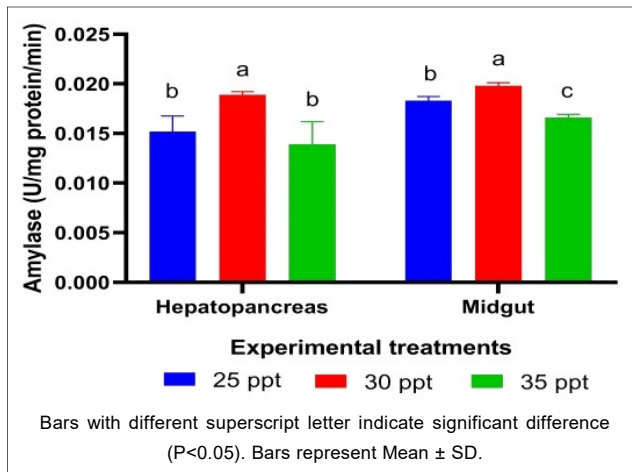


Fig 1: Amylase activity in the hepatopancreas and midgut of *Scylla serrata* reared at different salinities for each organ.

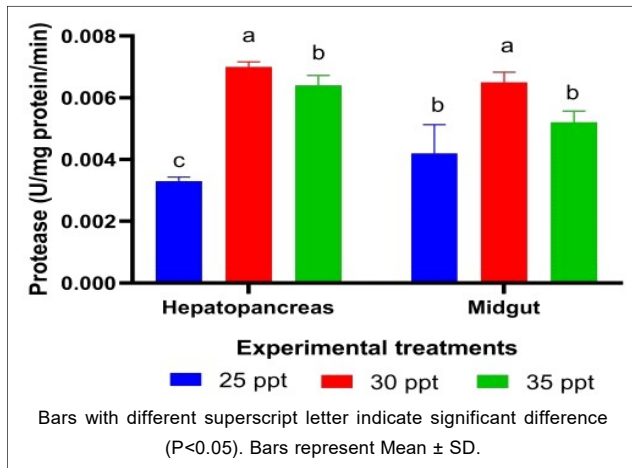


Fig 2: Protease activity in the hepatopancreas and midgut of *Scylla serrata* reared at different salinities for each organ.

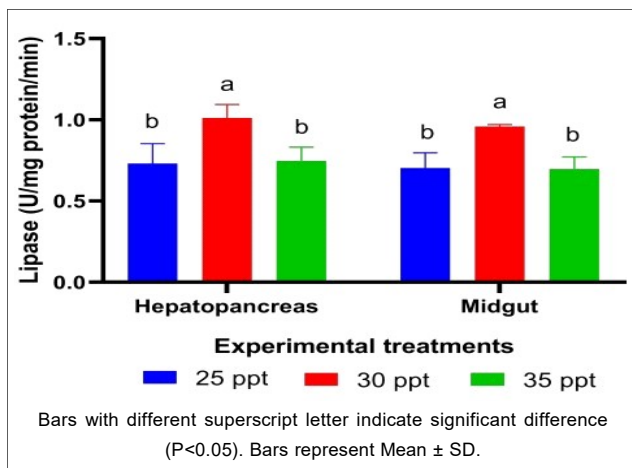


Fig 3: Lipase activity in the hepatopancreas and midgut of *Scylla serrata* reared at different salinities for each organ.

enzyme activities were recorded in mid gut of mud crab at 30 ppt salinity. The enhanced enzyme activity at 30 ppt may be attributed to the species physiological adaptation to this salinity, which promotes better dietary digestion and nutrient utilization. These results align with those of Chamchuen *et al.*, (2014), who reported increased lipase and protease activities in response to protein- and lipid-rich diets. Similarly, Asaro *et al.* (2018) found that amylase activity in crabs increased with higher carbohydrate levels in the diet. Wang *et al.* (2022) also supported the idea that digestive enzyme expression can adapt to dietary and environmental factors, including salinity.

While our findings are consistent with previous studies, slight differences in enzyme activity levels may arise due to variations in experimental design, species or subspecies studied, diet composition and acclimation periods. These factors could influence the crab's physiological responses and lead to differences in the magnitude of enzyme activity observed across studies. Nonetheless, our results confirm that a salinity of 30 ppt optimizes digestive enzyme activity in *Scylla serrata*, contributing to better feed utilization and potentially improved growth performance.

Histology

Histological analysis was conducted to assess how varying salinity levels affect internal organs, particularly focusing on the hepatopancreas and midgut, as these are critical for digestion and absorption in crustaceans. The present study did not find any deformity in the mid gut of *S. serrata* reared at different salinities (Fig 4). In contrast, *S. serrata* reared at different salinities showed deformities in the hepatopancreas. Among all the treatments, 30 ppt salinity treatment showed many tubular lumen contained sloughed transformed microvillar structure of hepatopancreas. Many tubular lumen were narrowed and blister cells were less observed in T1 (25 ppt) and T3 (35 ppt) (Fig 5).

At 25 and 30 ppt, we observed fewer blister cells and narrowed tubular lumens, indicating mild structural stress. However, at 35 ppt, more severe changes were evident, including sloughed epithelial cells and disrupted microvillar structures within the tubular lumen, suggesting cellular damage and reduced nutrient absorption capability. These findings are consistent with previous studies, such as Rosas *et al.* (2001), which also highlighted the hepatopancreas as a sensitive organ in crustaceans under salinity stress. The differences in severity observed between our study and others could be due to species-specific tolerance, experimental conditions, or the duration of salinity exposure. Such variations emphasize the importance of species-specific physiological responses and environmental factors when interpreting salinity tolerance.

An osmotic imbalance brought on by exposure to suboptimal salinity can cause histological damage in digestive tissues, such as tubular deformation and epithelial sloughing (Zhu *et al.*, 2018). In line with studies by Genodepa (2018), who noted that the hepatopancreas is the principal organ responding to osmotic and metabolic stress in crabs,

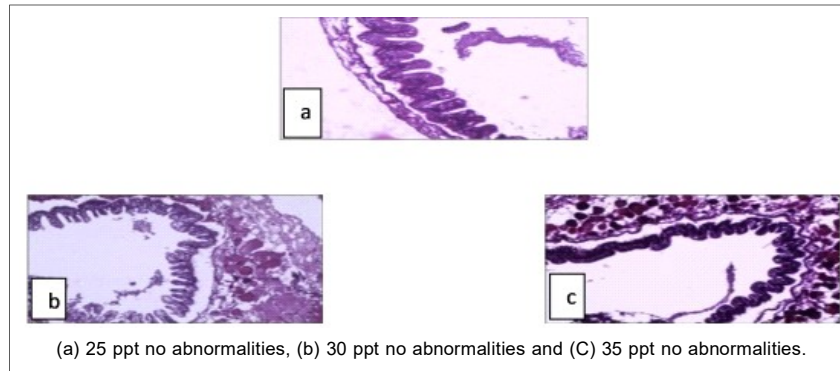


Fig 4: Micrographs of midgut of the *S. serrata* at 20X magnification.

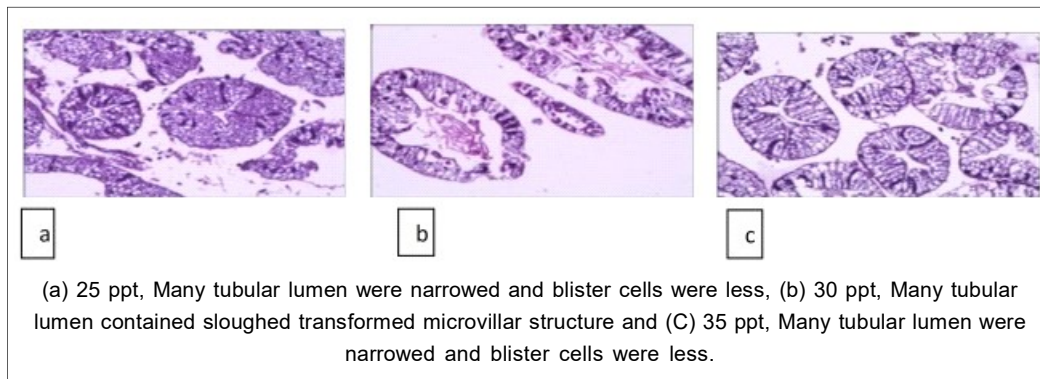


Fig 5: Micrographs of hepatopancreas of the *S. serrata* at 20X magnification.

the lack of midgut alterations implies that this region is more resilient or less directly impacted by external salinity variations. According to concurrent physiological research, the observed structural damage at 35 ppt is also associated with decreased digestive enzyme performance (Zhang *et al.*, 2021). The reduced ability to digest food at this salinity level may be explained by the sloughing of microvillar structures, which may hinder the absorption of nutrients.

CONCLUSION

The present study highlights the critical role of salinity in *Scylla serrata* aquaculture, identifying 30 ppt as the optimal condition for the grow-out stage. At this salinity, crabs exhibited significantly higher weight gain and average daily gain, supported by enhanced digestive enzyme activities (lipase, protease and amylase) and stable water quality. Histological observations further confirmed minimal stress, with normal hepatopancreas and midgut structures. In contrast, higher salinities such as 35 ppt caused severe tissue disruptions, including microvillar damage and epithelial sloughing. Overall, 30 ppt provides the best balance for growth, nutrient utilization and physiological health, offering evidence-based guidance for sustainable mud crab farming.

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Conflict of interest

The authors declare that they have no conflict of interest.

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