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Tidal periodicity of molting in giant mud crab, *Scylla serrata* (forskål, 1775)

Darwin C Biag [1, 2, *], Antonino B Mendoza Jr [2, 3], Renan U Bobiles [2, 3], Alex P Camaya [2, 3], Skorzeny C De Jesus [2, 3] and Plutomeo M Nieves [2, 3]

[1] *Camarines Norte State College – Institute of Fisheries and Marine Sciences, Mercedes, Camarines Norte, Philippines.*
[2] *Bicol University Graduate School, Legazpi City, Philippines.*
[3] *Coastal Resources Management Unit, Bicol University Tabaco Campus, Tayhi, Tabaco City, Philippines.*

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Abstract

Molting is the most crucial phase in the life cycle of mangrove crabs, leading to mortality in various culture systems. As a result of this exigency, this study was conducted to offer a cue in molting using the tidal cycle as one of the visible events in a natural context. Monitoring was carried out at every 1-hour interval (24 hours) day and night for 57 days to check for molting while taking into account key environmental elements such as tidal cycles, water current speed, and flow rate. The results showed that 70.6% of molting happened during high tide, with 93.8% of it occurring at night and 6.3% during the day. By contrast, only 29.4% occurred during low tide, showing a significant difference (T-test = 0.011, $p < 0.05$) between mean molts. Hence, the highest molting rate (88.2%) was observed between tidal episodes between the neap and middle cycles, with the middle tide (55.9%) occurring at 12.4±0.9 m/s, neap tide (32.4%) occurring at 16.8±2.0 m/s, and spring tide (11.8%) occurring at 9.5±1.2 m/s. Molting occurs at high tide because crabs' rheotactic behavioral responses to incoming new water help the crab's body fill with air and water throughout the molting process. Mangrove crabs employ their tidal periodicity of molting as a defense strategy against potential predators and mortality, and it can be used as a molting indicator in many culture systems.

Keywords: Tidal cycles; Water current; Molting schedule; Indicators; Mangrove crab

1. Introduction

Mangrove crabs are one of the most economically valuable fishery products [19], and *Scylla serrata* is the preferred species [17]. Among the other species, it is the largest and most widespread [13], [1]. Locally, it's called "Alimango," "Putian," or "Bulik," and it's known as a species champion because of its fast growth, high demand, and market value. Interest in crab farming has increased because of the growing market and international demand. Its high economic value and potential as an export commodity have expanded both the capture and culture of live crab [11]; becoming one of the livelihoods of many Filipinos in coastal areas, specifically in Southern Luzon (SL) and Visayas [7]. On the other hand, the province of Camarines Norte located in SL has long been recognized for its enormous mangrove crab resources due to its strategic location at the center of three key fishing grounds: Lamon Bay, San Miguel Bay, and the Philippine Sea. The province is well-known throughout the country as a reputable source and exporter of high-quality wild seed and live mud crabs recently called mangrove crabs. Traditionally, mangrove crabs have been harvested from the wild as a source of food and income. Various sizes, including crab instars, juveniles, and adults, are collected and marketed locally and outside of the province. Some are raised in fishponds for grow-out and fattening. However, during the COVID-19 pandemic, shipping of live mangrove crab was also disrupted owing to tight enforcement and lockdowns, resulting in a large supply and a price decrease that was double the regular price. Adult-sized crabs have been priced between 200-250 pesos per kilogram at public markets, crab buying, and landing stations during the early and late outbreaks. While

* Corresponding author: Darwin C Biag
Camarines Norte State College, Institute of Fisheries and Marine Sciences, Mercedes, Camarines Norte, Philippines.

60-gram juvenile crabs were around 5-10 pesos, 80-150-gram were worth 15-25 pesos each and were significantly cheaper throughout the season. Because of this, there has been a rise in interest in aquaculture businesses, such as crab nurseries, soft-shell crab farming, and fattening in crab box containers due to oversupply, as an alternative to exporting live crab. The increasing pressure on wild crab harvest to meet demands in both aquaculture and live transport of mangrove crabs has increased soon after entry bans and lockdowns are lifted. Currently, aquaculture technologies applied in crab farming in Camarines Norte are the nursery of collected crabs from megalopa – juvenile stage in fish ponds within mangrove areas; crab fattening; polyculture with one to three other species like milkfish, tilapia, grouper, shrimp, or prawn; and recently, soft-shell crab production.

Hence, to grow crabs, a periodic shedding of the exoskeleton is required [17]. The vegetative growth (somatic growth) of the animal is regulated by a special process called molting, which is the shedding of the old exoskeleton and the synthesizing of the new exoskeleton, required for the ever-growing body size [16]. According to Josileen and Menon [12], molting happens 4-6 times from the zoea to the megalopa stage in portunid crab larvae, while juveniles molt more than ten times from the instar stage before becoming mature adults. However, cannibalism during molting has been recognized as one of the major issues affecting mud crab culture. Mirera and Mtile [15], stated that predation after molting is one of the main causes of mortality aside from molting failure and sudden death during molting. Furthermore, research has shown that tidal cycles have a substantial impact on molting schedules and mortality. Mirera and Mtile [15], stated that molting was a cause of mortality for cultivated crabs due to predation, injury, and other physical stressors. They also found that 59% of molting happens during neap tides and 3% during an earlier transition period of spring tides, with all molts happening at high tide. However, this study was conducted in drive-in cages rather than in crabs' natural habitats of fish ponds or brackish water environments. The speed and flow rate of water produced by various tidal cycles were not quantified or recorded even in other studies. There has been a minimal investigation into the influence of constant changes, such as the tidal cycle, on molting. Most studies and literature define tidal events by focusing on low and high tide cycles without taking into account the mid-way cycle or transition between high and low water marks. As stated by Hockley et al. [10], water currents generate behavioral variability within a species, generally in response to velocity. This research assumes that a multitude of environmental factors can influence molting, including tidal current at different tidal settings, water volume, and current speed between different tidal cycles: neap, middle, and spring tide cycles.

Thus, to improve current farming practices, micromanagement corresponding to the tidal periodicity of molting is considered necessary, specifically to meet the demands of farmers and governments seeking to promote crab aquaculture enterprises in the new normal, particularly in grow-out ponds, aqua-silviculture, polyculture, nursery areas, and in soft-shell crab farming facilities. Tidal periodicity in molting is one key aspect to improving survival rate, preventing mortality, and identifying molting schedules in soft-shelling facilities and different culture systems. Identifying the tidal periodicity in molting will aid in solving the present problem in the monitoring of molting in soft-shell crab farming, in addition to adopting suitable techniques to avoid mortality during molting in different rearing systems (grow-out ponds, nursery, and polyculture areas). As a response to this need, this study was carried out to analyze the tidal periodicity of molting in giant mud crabs (*Scylla serrata*) to provide technical knowledge and empirical information on molting while taking into account important environmental factors such as the speed of water current and flow rate produced by different tidal cycles.

2. Material and methods

This research used primary data by conducting an onsite experiment to determine the tidal periodicity of molting in giant mud crabs (*Scylla serrata*). The experiment was carried out from May 7–July 2, 2021 (57 days) at the brackish-water fishpond of Camarines Norte State College—Institute of Fisheries and Marine Sciences, located at Mercedes, Camarines Norte, Philippines. A total of 90 mangrove crab specimens (*S. serrata*) weighing 80-100g were randomly stocked in individual crab trays measuring 16.7 cm x 16.7 cm x 12 cm. Bamboo floating pontoons were made to serve as holding frames for crab trays. Crab pontoons were positioned inside the center of a 2000 m^2 brackish-water fish pond. Strategically, it was constructed at the middle part of the fish pond, facing the gate with designated distances at 1 m intervals to receive an equal distribution of new water during high tide. Three modules were fabricated measuring 3m x 1m, which were divided into three (3) segments. Each segment held ten (10) crab trays as shown in Figure 1.

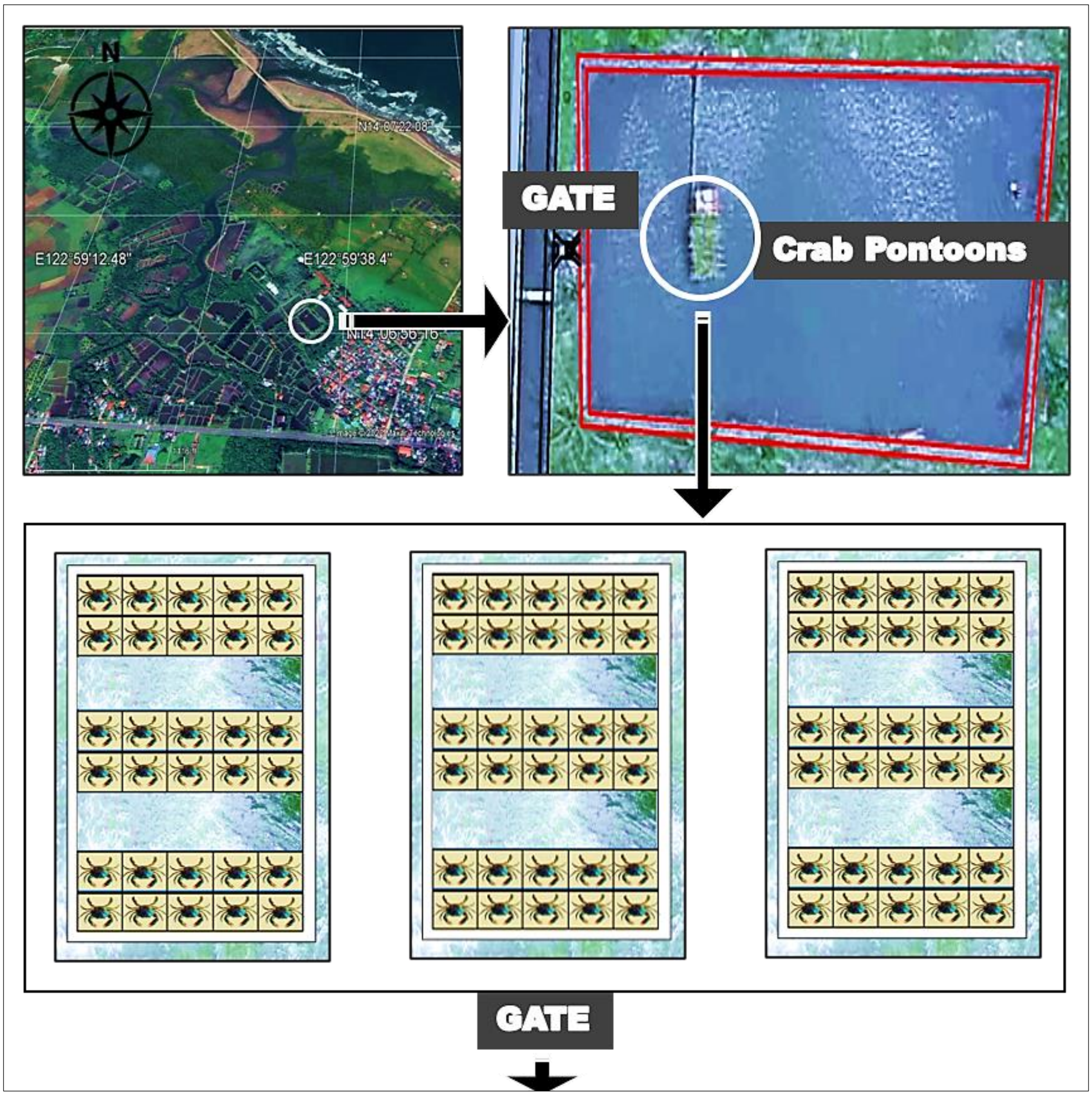

Figure 1 Location map of Camarines Norte State College – Institute of Fisheries and Marine Sciences, showing an aerial view of the experimental set-up of the study

2.1. Acclimation of stock

Before stocking, crabs were placed in a plastic basin and gradually sprinkled with pond water during the acclimation. After such, crabs were stocked in individual crab containers which served as a holding area to give enough time to adapt to the new environment. Two days after, weak and dead crabs were removed and healthy crabs were selected and subjected to body measurements before stocking. This method was done to ensure the high survival of crabs in soft-shell crab production systems and prevent mortalities due to capture stress.

2.2. Pond water management

Pond water management was done by opening the gate/slab during high tide and closing it during low tide. Water exchange was done at every high tide to let entry of new water. Other parameters like water quality were constantly monitored such as salinity, temperature, pH (acidity), dissolved oxygen, and water depth. Gates screen was brushed daily to remove debris that may cause clogging of water during pond freshening. Dike and slab leaks, were checked from time to time to prevent water loss.

2.3. Feeding

Crabs were fed with pony fish and telescope snail meat daily. The feeding ration was given at 5% based on the stock's average body weight. Individual feeding of crabs in each container was done twice a day: early morning (25%), and late afternoon (75%). Feeding was done between 8:00 AM – 9:00 AM (morning), and between 5:00 PM – 6:00 PM (afternoon).

2.4. Monitoring of molting and tidal cycles

For 57 days, monitoring at every 1-hour interval (24 hours) day and night were done to check for molting. Every 1-hour interval, crab trays were individually checked by towing the crab pontoons under the bamboo bridge recording the occurrence of molting and taking notes of tidal periodicity in terms of high tide and low tide cycles and tidal events (neap, middle & spring tide). Tidal episodes; neap, middle, and spring tides were determined using the tidal calendar following Global Tides version 4.2.19 mobile application to record dates and number of different tidal events during the culture period.

2.5. Water speed and flow rate (CFS) measurements

Water current is defined as the rate of movement in the water which includes speed and direction [21]. High and low tide cycles were recorded by observing the in and out water flow direction during high tide and low tide in the gate. High tide and low tide cycles were noted by observing the water direction and recording the speed and flow rate of the water current. The speed of the water current that flows through the gate was measured using an improvised water current meter. A line transect was deployed to measure and establish distance from the pond gate up to 3m where the effective current flow was observed. A meter stick was placed at a 3-meter distance to serve as an end-point in measuring water current and as a depth gauge to measure water height. An improvised floating device made of rubber was used as a water current meter to record the surface velocity/ speed of water with the aid of a stopwatch. Since water at the edges of the gate flows more slowly and sometimes moves in the left and right direction creating whirlpools due to its bank's friction, the floating device was dropped at the center of the gate during sampling, recording water speed in 3 trials. Speed/velocity (in meters per second) was calculated by dividing the distance by the computed average time (in seconds).

The speed of water current was calculated using the formula:

$$s=d/t,$$

Where;

s = speed;
d = distance travelled
t = time elapsed.
The flow rate in cubic feet per second (CFS) was calculated using the formula:

$$\text{Flow (CFS or ft3/s) = area (ft2) x velocity (ft/s).}$$

To calculate the CFS, first, the designated distance of 3m was converted into feet and then divided by the average speed to get water velocity in ft/s. Second, the computed velocity (ft/s) was multiplied by 0.85 (a correction factor) to estimate the average water velocity. The Area (ft2) was calculated by multiplying the width (ft) of the pond gate by the average depth (ft) of water.

2.6. Physicochemical parameters

Water quality parameters like salinity, pH (acidity), dissolved oxygen, and water depth were also recorded at the center of each crab pontoon. A smart sensor digital salinity meter (handheld ATC salinometer) or salinity tester with 0.00ppt-9.99ppt and 10.0ppt-50ppt measuring range for seawater was used to record the salinity level of the pond water. Milwaukee digital pH meter was used to determine water acidity and alkalinity. Dissolved oxygen of the pond water which is important for the life-sustaining process was recorded using a dissolved oxygen meter. During monitoring, other instruments were used are a water-resistant pocket-size wall clock to record the time of the high tide and molting; plastic meter sticks - established at sampling stations to measure and monitor water depth, and a waterproofed flashlight - used during night monitoring and data collection. The light was only used during monitoring to check for molts, it is turned off after to preserve the dark environment of crabs at night-time.

2.7. Statistical analysis

This study used descriptive techniques in constructing tables of means, frequency, standard deviation, standard errors, percentages, and cross-tabulations. While inferential statistics were used to highlight differences between molting relative to high and low tide cycles using a T-test. Molting frequency of crab relative to tidal events (neap, middle, and spring tides), water quality results were computed and presented using frequency distribution models.

3. Results and discussion

3.1. Molting activity of mangrove crab

As the growth proceeds in crabs their size increases, that's why they need to undergo a process called molting to get rid of their old exoskeleton since their shell cannot expand. In this study, crabs exhibit less movement and feeding behavior during the pre-molt stage/pro-ecdysis. A development of v-shaped cracks on the inner part of the two chelipeds happens [15]. Visible shell breaks can be also noticed on both ventral sides around its carapace. To make a progressive crack around the shell during ecdysis, crabs create a strong body contraction where it rests in an upright position, extending both cheliped and open pincers. The body is filled with air and water through the mouth to expand and create breaks around the old carapace. Muscle contraction and air filling in crabs caused the body to swell so that the old exoskeleton cracks along the ecdysteroid sutures [9].

The exuviation/casting off of the old carapace begins from the mid-posterior carapace (breaking point) – found between the two swimming legs/5th pereiopod up to the anterior side, creating symmetrical progressive cracks around the carapace. Opening and separation of the old carapace from the ventral body leave only the mid-anterior part of the shell, weakly attached along to the mouth. After the posterior area is completely opened up to the anterior side, the old carapace overlaps with the new carapace exposing its lateral spines. At this stage, crabs halt from absorbing water then wriggle out "body pull-out" and retract their limbs (cheliped, walking, and swimming legs) out of the old shell. The swimming legs/5th pereiopod first protrudes out, followed by the walking legs (4th, 3rd, 2nd pereiopods) and chelipeds/1st pereiopod in order (Figure 2).

The strong force applied by crabs during the retraction of limbs out of the old shell is the most critical stage in molting. Because, as the crab wriggles out, reversely pushing its soft body, appendages are sometimes stuck inside the old shell, resulting in limb loss (e.g., chelipeds, walking legs, or swimming legs). That's why there were newly molted crabs that can be found with incomplete appendages after molting. To prevent limb loss during this stage, crabs maneuver their positions using their swimming and walking legs to find enough space to execute body pull-out. After such, crabs persistently push off the old shell using their chelipeds or walking legs. Mostly, both chelipeds are used to push the old shell to support pull out of the stuck walking legs, while in case a single cheliped is stuck, the old shell is solely pushed by the other cheliped together with its walking legs.

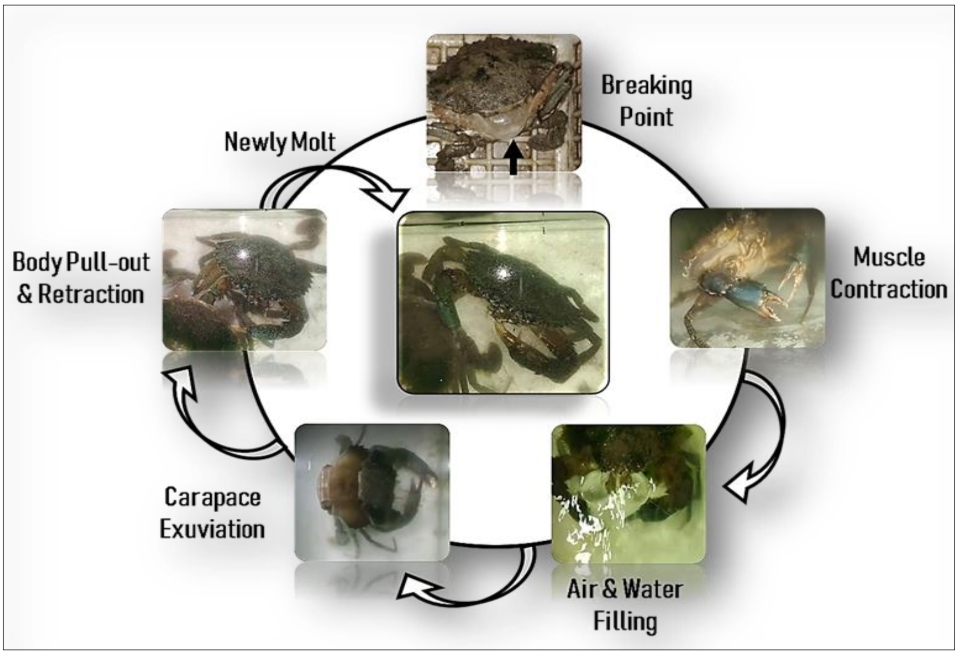

Figure 2 Schematic representation of mangrove crabs' molting processes in the soft-shelling facility

After a successful molting crabs become inactive and possessed less movement activity as the exoskeleton is still soft. The new exoskeleton can be considered “truly soft”, characterized by high water content and low levels of calcification [22]. According to Mirera and Mtile [15], at this stage crab’s exoskeleton are delicate due to its “sponge-like” soft muscle that resembles like to be a thin membrane. Henceforth, the shell starts to harden within hours [17]; and as the exoskeleton hardens crabs started to increase body movement and activity after it regains muscle control.

3.2. Tidal periodicity in molting

A total of 75.6% (68) molting was recorded out of the 90 stocks constituting 70.6% during high tide (inflow) and 29.4% during low tide (outflow). The highest molting during high tide was recorded at night time (93.8%) compared to daytime (6.3%) as shown in Figure 3. Mean molts between high and low tide cycles showed a significant difference (T-test = 0.011, $p < 0.05$). Tidal cycles can be used as a cue in predicting molting and mortality prevention caused by cannibalism. During high tide, the water stream from the gate running to the crab pontoons assists in the air and water filling of crabs, allowing for a speedier molting process. Crabs are also more active at night and during high tide, and they like to scratch and claw their way inside containers. Mangrove crabs can also be categorized as rheotactic because of their active behavior during water inflow. Because of these factors, water current during high tide may make crabs more active and can be regarded as a significant environmental element in molting.

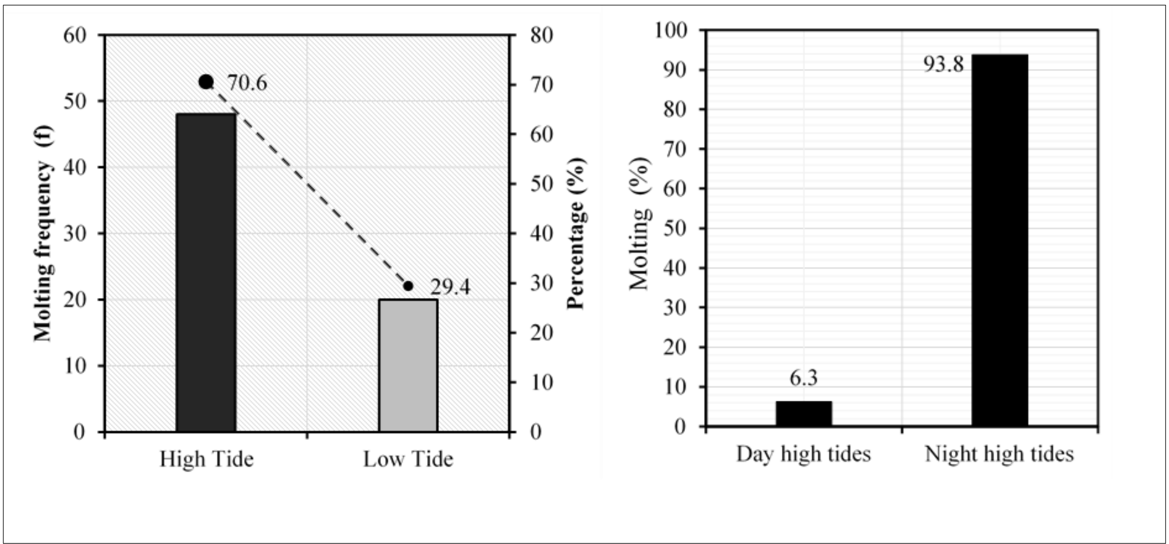

Figure 3 Bar graph illustrating molting between cycles of high tide and low tide (left chart), depicting molting during the day and at night time high tides (right chart)

3.3. Incidence of molting between tidal episodes and water current

Table 2 showed the total frequency of different tidal episodes, speed of water current, and percentages of molting. The total number of days per tidal event consisted of 11- neap tides, 34 - middle tides, and 12 - spring tides based on Global Tides application. A high incidence of molting per tidal episode and speed of water current (SWC) was observed at middle tide (55.9%) at a mean SWC of 12.4±0.9 m/s, followed by the neap tide (32.4%) at 16.8±2.0 m/s SWC, and spring tide (11.8%) at 9.5 ±1.2 m/s SWC. The overall incidence of molting (88.2%) can be observed between the neap and middle tidal cycles at a mean SWC of 12.4 – 16.8 m/s.

Table 2 Summary of means and standard error of water current velocity; percentage of molting, *n*=68, and the number of tidal episodes

Tidal Episodes	No. of Days	x̄ Speed of Water (m/s)	Molting % (n=68)
Neap	11	16.8 ±2.0	32.4
Middle	34	12.4 ±0.9	55.9
Spring	12	9.5 ±1.2	11.8

Given that molting occurs at high tide or water entry, the water current has a substantial impact on molting. However, the speed of water and flow rate varies depending on the tidal episodes: neap, middle, and spring tides. Most of the time, the term "middle tide" is not defined in the literature and studies. Tidal events comparisons are frequently centered on high and low tide cycles. However, middle tide, which occurs halfway between neap and spring tides, can cause large fluctuations. Because, as demonstrated in this study, each tidal cycle produces a variable surface velocity and volumetric flow rate of water current, leading to a varied molting frequency. Moreover, the volumetric flow rate in cubic feet per second (CFS) varies between different tidal cycles. The mean flow rate during a spring tide (5.42 ft^3/s) is higher compared with the middle (3.67 ft^3/s), and neap tide (1.66 ft^3/s). The volumetric flow rate of water during neap tide is increased by 2.01 ft^3/s at the middle tide and 1.75 ft^3/s during spring tide. Fastest speed of water current (m/s) was observed during neap tide as the volume of water is lower, while water speed is slower as the water volume is higher during middle and spring tides (Figure 4). The occurrence of high tide was recorded at an average of every 12.5 hours intervals. While recorded mean pond water height around the crab pontoons at every 1-hour interval was 61.3 cm ranging from 38.0 cm minimum and 124.3 cm maximum level. The total tidal episodes frequency across the study period consisted of 4 spring tides, 4 neap tides, and 8 middle tides cycles as shown by the highest and lowest water level mark in Figure 5.

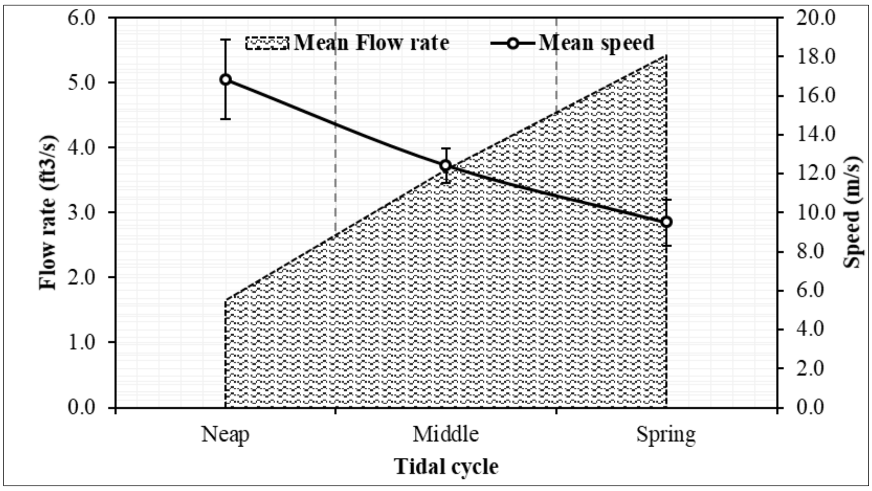

Figure 4 Mean flow rate and speed of water entering the pond gate during different tidal episodes

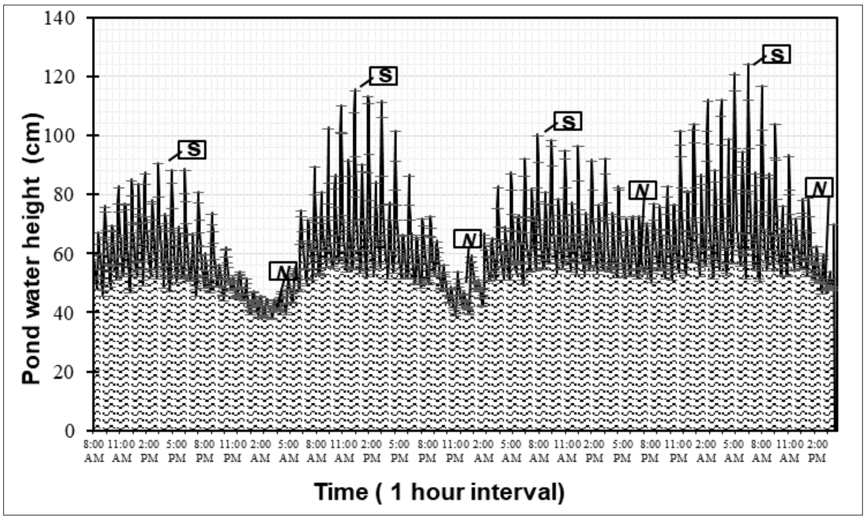

Figure 5 Time series graphical presentation of mean pond water height in 57 days culture period. Letter S represents the occurrence of spring tides while N is represented by the frequency of neap tides. Middle tides are halfway every spring and neap tides cycle

Depending on the tidal event, the surface velocity or speed of water flow is controlled by the volume of water entering the pond. This has an impact on the volumetric flow rate of pond water, which is defined as the volume of water moving through a particular surface per unit of time [24]. The unit of measurement for moving water is cubic feet per second (CFS) [5]. Simply put, varied molting frequencies are caused by the volume and speed at which water flows through the pond's gate at cubic feet per second (CFS). These variations are driven by tidal episodes which cause the volume and height of water entering the pond to fluctuate as the tides vary. The volume and height of the water inside the pond were principally influenced by the size and height of the incoming water. Concerning these events, the middle tide had the highest molting frequency, followed by the neap tide and spring tide. Molting was much more frequent during the neap tide, which has the fastest water current speed, compared to the spring tide, which has a slower pace. However, molting occurs best at the middle tide or mid-way water level between the neap and spring tide transitions, when the water current is at moderate speed. It should be emphasized that tidal cycles serve as a triggering factor for molting and aid during the body air and water filling process. Thus, tidal periodicity during molting is one defensive strategy used by crabs to avoid predators. As a result, when there is a shelter nearby, the frequency of molting increases [6]. According to research, the time of molting was higher at night, particularly during night high tides [4], [15]. Because the molting itself causes mud crabs vulnerable to cannibalism or predation [8]. With this, the water level in natural brackish-water fishponds decreases during the neap tide, exposing mud crabs to high temperatures and potential predators. While most species (both wild and farmed) including mangrove crabs within the fish ponds are more active during spring tide, leading to higher vulnerability for newly molted crabs. Furthermore, spring tides are formed by the alignment of the earth, moon, and sun at the new or full moon, while neap tides are caused by the sun and moon aligning at right angles to the earth during the first and third-quarter phases [14]. Spring tides occur during the new and full moons when predators and other aquatic species are at large attributable to their phototactic activity.

3.4. Effect of the water current on the condition of crabs

Figure 6 showed the condition of crabs (picture A-C) stocked at the back 3rd layer of crab pontoons that received a lesser speed of water current during high tide or inflow of water in the gate, resulting in the accumulation of dirt and growth of filamentous green algae on the carapace and around the body exposed to sunlight. Crab in pictures A (Top view), and C (Front view) showed the actual air and water filling of crab during molting preparations to expand. Picture B is the rear view of the crab showing the breaking point of the old carapace on the mid posterior carapace, while picture D showed a newly molted crab with a soft and clean exoskeleton after a successful molting. As a result, heavy growth of algae around the body prevents crabs' routine movement, preening activity, and feeding and obstructs molting.

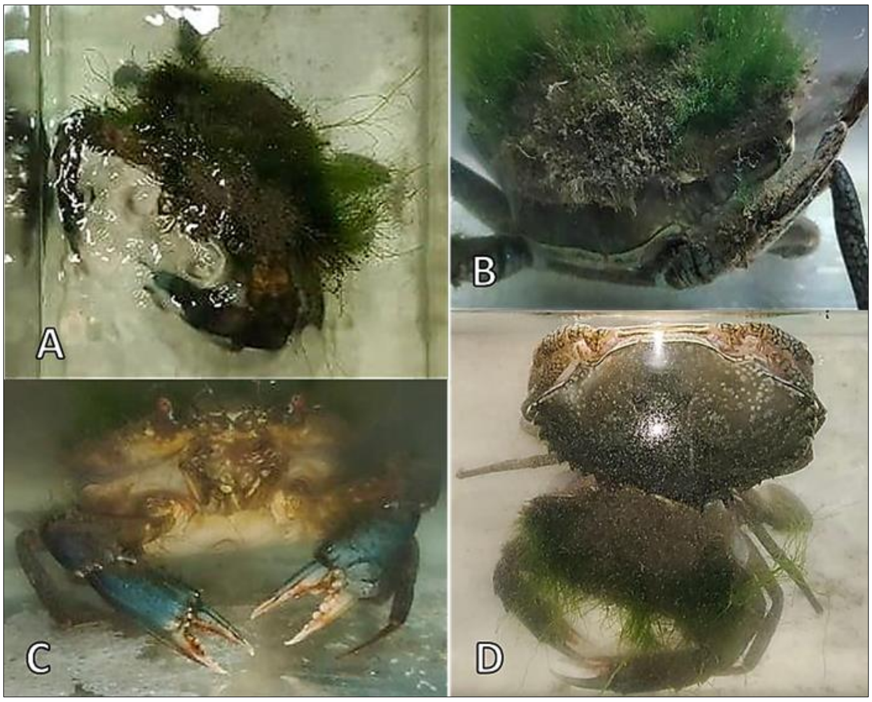

Figure 6 Crab condition on the third layer of crab pontoons, with visible growth of green algae around the body due to slower water current

3.5. Physicochemical parameters

Results showed that external factors that affect molting include tidal cycles and water currents. Aside from these, another important parameter is the water quality which showed the condition of the environment throughout the cultural period. Studies showed that successful management of fish ponds requires an understanding of water quality [2] since water quality in the fish ponds changes continuously which often affects the optimal levels of physical and biological characteristics [23]. Physicochemical are directly related to their production and are one of the greatest concerns for fish farming to consider [20]. Hence, in this study, the analysis of physicochemical parameters during the culture period was revealed to be at optimal levels consisting of mean and standard error salinity of 30.88 ppt ± 0.11 ppt, pH levels of 8.23 ± 0.01 and dissolved oxygen level of 5.32 mg/L ± 0.08 mg L^1 (Figure 7). In addition, Shelley and Lovatelli [18], stated that pH in mud crab culture should be at 7.5 - 8.5 based on marine shrimp requirements, as little work has been done on mud crabs. In terms of salinity, mud crabs are found to be highly adaptable and can tolerate broad ranges of salinity from 1-56 ppt [1], with optimal growth at 10-25 ppt [18]. Dissolved oxygen was also said to affect the growth, survival, distribution, behavior, and physiology of fish and other aquatic organisms [3]. Hence, high levels of dissolved oxygen were observed at high temperatures during the day and tend to go down at night.

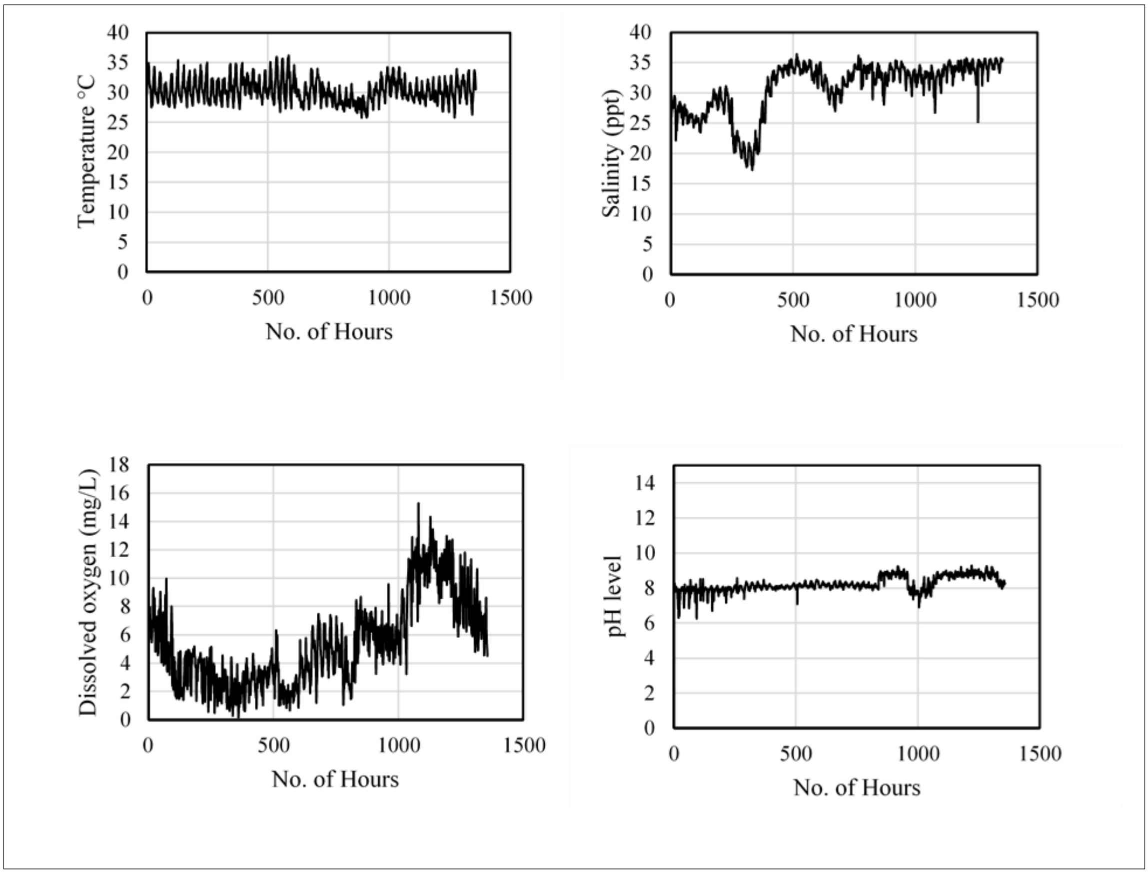

Figure 7 Graphical presentation of physicochemical parameters at every 1-hour interval sampling in 57 days culture period

4. Conclusion

Based on the results and findings, tidal periodicity of molting in giant mud crab (*Scylla serrata*) happens during high tide and middle tide cycles. Molting happens at high tide because crabs exhibit a rheotactic behavioral response towards incoming new water which aids in the air and water filling of the crab's body during the molting process. The highest molting at middle tide is due to the moderate speed of water current and ideal water volume which is midway compared to drastic changes in water level and volume during the neap and spring tide cycle. The tidal periodicity of molting is a defense mechanism of mangrove crabs to prevent mortality and possible predators. Further, due to crabs' nocturnal behavior, tidal periodicity in molting accounts not only for the tidal cycle, speed, and volumetric flow rate of water but may also include photoperiod as visibility at nighttime varies brought by different moon phases associated with the different tidal cycles. Overall, the tidal cycle and water current can be used as an indicator of tidal periodicity in molting in grow-out and soft-shell crab farming. Molting can be possibly induced by simulating water speed and flow rate in an outdoor and controlled environment.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have no conflict of interest to declare. All co-authors have seen and agree with the contents of the manuscript.

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