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SHORT COMMUNICATION

# Effects of Temperature and Diurnal Cycle in the Molting Schedule of Mangrove Crab, *Scylla serrata* (Forskål, 1775)

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## ABSTRACT

Soft-shell crab farming is gaining popularity since all crab parts can be eaten with almost no discards. However, its biggest challenge is the tedious monitoring every 4-hour interval to check for molting. A total of 90 mangrove crabs weighing 80-100 g were stocked in individual crab trays, recording 24-hour water temperature and incidence of hourly molting for 57 days to provide another basis for monitoring and cues in the molting schedule. Results showed that molting (82.24 %) happens at optimum temperature ($T_{opt}$) levels, between 27–31°C, with a high incidence of molting (85.29 %) at nighttime when the water temperature is lower and within the $T_{opt}$. Findings suggested that aside from 4-hour intervals, monitoring of molting for soft-shell crab farming is recommended at nighttime and early morning.

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Soft-shell crabs are among the top emerging fishery products with huge export potential (Basyuni et al. 2020). Soft-shelled crabs are referred to as "luno" in the local dialect, which means newly molted with a soft body or shell. All parts of a soft-shell crab can be eaten when cooked (Quinitio et al. 2015). It is also considerably more expensive, ranging from PHP 1,500 to 2,000 per kilogram, compared to hard-shell crabs, which cost only PHP 250-500 per kilogram. The country's export market includes mainly Singapore, Taiwan, Hongkong, and China (Peralta and Chan 2017). Southeast Asian countries (Myanmar, Vietnam, and Thailand) produced great quantities of soft-shell crabs, sold to local restaurants and exported overseas with a price range of USD 10–15 $kg^{-1}$ or higher (Aquino 2018). In the Philippines, soft-shell crab farming is a new technology that started in 2012 at the Southeast Asian Fisheries Development Center (SEAFDEC-AQD) using 60–100 g crabs, adopted from the individual culture system of Thailand and Myanmar (Quinitio et al. 2015).

In 2018, under the National Crab Production and Livelihood Program (NCPLP), soft-shell crab production technology was introduced in the Bicol Region through the effort of the Bureau of Fisheries and Aquatic Resources (BFAR) Region V. BFAR conducted a series of extension training through partnerships with State Universities and Colleges (SUCs) in Bicol Region. However, the common problem encountered by crab farmers was the constraints in monitoring 4-hour intervals due to asynchronous molting, resulting in low adopters. Unlike other crab production systems, soft-shell crab farming requires tedious feeding and monitoring of 4-hour intervals to check for molting (Rahman et al. 2018). It also involves sorting 60–100 g immature mud crabs for stocking (Quinitio et al. 2015; Aquino 2018). Checking for molts every 4 hours, day and night, and only finding a few molted crabs is a labor-intensive and cost-ineffective crab production system. Generally, it takes around a maximum culture duration and monitoring of 60 days or higher. The pontoons are restocked with a new supply when the previous molting cycle is finished. Due to the asynchronous nature of the molting process, obtaining a sufficient amount of soft-shell crab items for marketing takes some time. Because of this, the usage of freezers requires more effort and costs more money. Individual feeding and monitoring, as well as the danger of death, escape from crab trays, and poaching, increase due to lengthy procedures. Soft-shell crab farming technology has few technology adopters and no established regional local markets. Most aquaculture operators prefer engaging in the usual farming practices, such as grow-out and crab-fattening cultures, which have a faster turnover than soft-shell crab production. This is why soft-shell crab farming technology is not as widely adopted as other aquaculture practices.

Nevertheless, soft-shell crab production has the potential to be a promising technology due to the fact that all parts of the crab can be consumed with almost no discard. Its popularity and market demand can be increased by refining the technology specifically in terms of molting and monitoring. Obtaining a significant number of soft-shelled crabs and reducing labor effort can be achieved through proper monitoring. A potential solution to overcome limitations is to comprehend the molting schedule and identify molting indicators that can serve as cues for monitoring. Studies have shown that molting is a complex process, affected by a range of environmental cues (Loeb 1993), growth and reproduction (Chang and Mykles 2011), and a range of factors such as temperature, photoperiod, nutrition, and eyestalk ablation (Hosamani et al. 2016). Molting is also controlled by a molt-inhibiting hormone (MIH) and ecdysteroids, where MIH inhibits the synthesis and secretion of ecdysteroids by the Y-organ, resulting in molt suppression (Katayama et al. 2003). The Y-organ (YO), or molting gland, is the source of steroid hormone production and consequent molt cycle regulation in decapod crustaceans and is responsive to both external environmental and internal physiological signals (Shyamal et al. 2014; Skinner 1985).

Hence, the most important single factor affecting the growth and development of crustaceans is the temperature (Azra et al. 2019). It is a dominant environmental factor that mediates the behavior, physiology, growth, survival, distribution, and recruitment of ectothermic animals in temperate and high latitudes (Stoner et al. 2010). Studies have revealed that temperature is an important factor in different crab species' growth, survival, and molting. Gong et al. (2015) stated that high temperatures decrease the molting duration, and extremely high temperatures affect the molting success in mud crabs (*S. paramamosain)*, where crab reared at 39°C resulted in death without molting. Under controlled conditions, growth using *P. pelagicus* instars in terms of carapace width increased at lower temperatures, while the inter-molt duration decreased with increasing temperature (Azra et al. 2019).

A previous study also showed that temperatures between 28°C and 32°C affected the survival and development of *P. pelagicus* larvae (zoeal and megalopa stages) (Baylon 2009). A higher survival rate of 77–80% was also observed at 28°C and 24°C, whereas the survival rate was 70% at 32°C. It is suggested that higher temperature results in greater activity and mobility, greater chances of fighting, and cannibalism, leading to higher mortality (Azra et al. 2018). It is not unexpected that an increase in temperature will reduce the inter-molt period (Azra et al. 2019). Yuan et al. (2017) found that juvenile *Eriocheir sinensis* reared at 28°C and 30°C grew significantly faster (shorter intermolt duration) than those reared at lower 18°C and 22°C temperatures. Compared to juveniles of another portunid crab species, *S. paramamosain* molting interval decreased with increasing temperatures between 20°C and 32°C. Warmer temperatures significantly decreased inter-molt periods of instar stages of lithodid crabs *(Lithodes santolla)* reared at water temperatures of 3-15°C (Calcagno et al. 2004). A similar pattern was observed in *Cancer magister* juveniles, in which warmer water temperatures (up to 15°C) shortened the intermolt periods compared to cooler water temperatures of 0–10°C (Kondzela and Shirley 1993). The factors behind the process might be generally associated with higher mRNA and protein levels of ecdysone receptor, EcR, in crabs at higher water temperatures (Gong et al. 2015). Higher water temperatures are also expected to increase metabolic processes in general and increase indirect molting. As to the molt increments, defined as the post-molt size compared to the size before molting for brachyuran crabs, they are also affected by water temperature. In general, increasing the temperature causes a reduction in molt increments (Hartnoll 2001).

Final carapace widths differed significantly with different rearing temperatures. A similar observation was noted in *C. magister* (Kondzela and Shirley 1993). The differences in intermolt periods can largely explain the difference in molt increments. It was found that *P. pelagicus* crabs grow faster at a higher temperature because of decreased inter-molt duration (Hicks and Johnson 1999). Higher temperatures promote molting, shorten inter-molt durations, and enhance the rate of carapace hardening. Rearing crabs at 32°C resulted in shorter inter-molt periods, time of molting, and more rapid carapace hardening of *P. pelagicus* crabs. It is suggested that decreasing the time for full hardening of the exoskeleton by increasing temperature will make the crabs less liable to cannibalism (Azra et al. 2019).

Related literature and studies revealed

that environmental indicators such as temperature influence crustaceans' molting, survival, growth, and reproduction. However, the temperature effect on molting, specifically in *S. serrata* has yet to be fully explored. Most of the studies focused on other portunid crabs and at early stages, evaluating different molting durations and changes in the life cycle. In addition, no research study shows empirical data and information on the molting schedule of mangrove crabs relative to temperature.

Information on the molting schedule of soft-shell crabs in relation to temperature is scarce. As a result, current monitoring methods require checking for molting every four hours. Considering that soft-shell crab farming is outdoors, it is important to study the effects of temperature, the primary environmental factor that can be influenced by photoperiod, season, and weather.

With this, if the molting schedule is identified, indicators may be used as a cue in monitoring to address labor-intensive monitoring requirements every 4 hours. Therefore, robust information on temperature and diurnal cycle effects relative to the molting schedule is deemed vital. Hence, this research work was carried out to provide empirical information that may be used as a basis for monitoring and technology improvement.

The experiment was conducted from 7 May to 2 July 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 soft-shell crab production set-up was used in the study. Crab pontoons were strategically constructed in the middle of the fish pond, facing the gate to receive an equal distribution and amount of new water during high tide, as shown in Figure 1. The pond shed structure adopted from soft-shell crab farming technology was constructed, which is made up of wood and bamboo materials. Coconut fronds were used as a shade to serve as a shelter from hot weather. The catwalk was installed and connected to the pond shed and dike for easy access during monitoring, harvesting, feeding, and data collection.

A total of 90 crab specimens weighing 80–100 g were stocked in individual crab trays. Crab trays used in the study came from BFAR. Bamboo floating pontoons to serve as holding frames for crab trays were fabricated, measuring 3 x 1 m with three segments. Each bamboo pontoon holds 30 crab trays, each to a total of 90 crabs. Distances between crab pontoons were maintained at 1 m intervals (Figure 2).

The mangrove crab (*Scylla serrata*), locally called in the area "alimango," "haniit" or "han-it," and "bulik" was used in the study. Specimens were

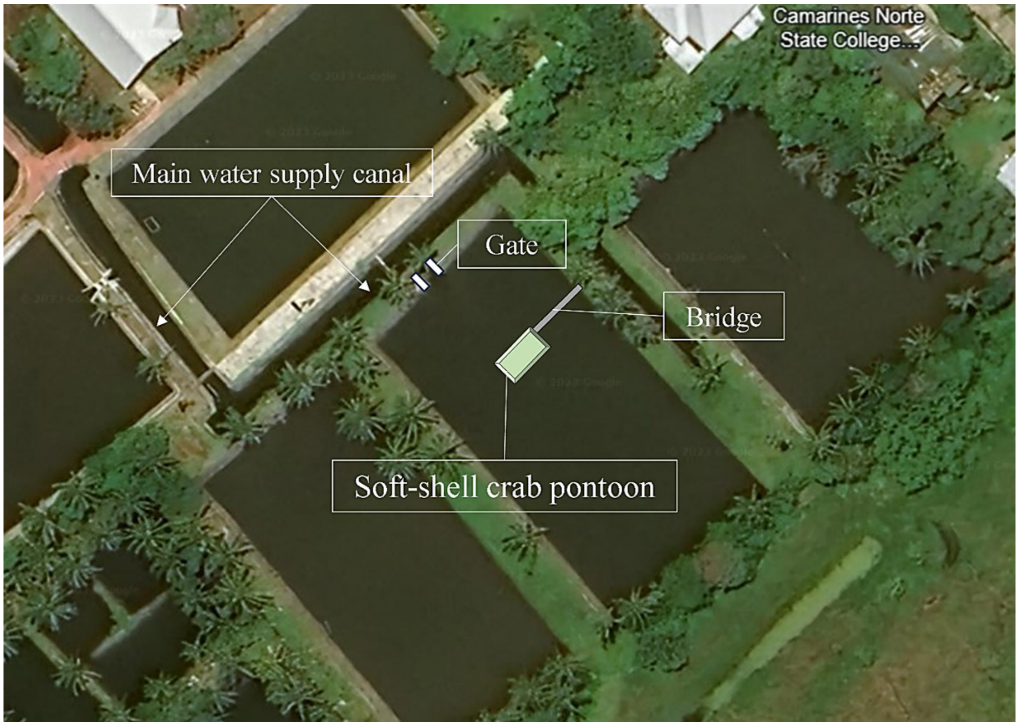

Figure 1. Aerial view of a 2000 m$^2$ brackish-water fish pond, showing the experimental set-up and location of the crab pontoons.

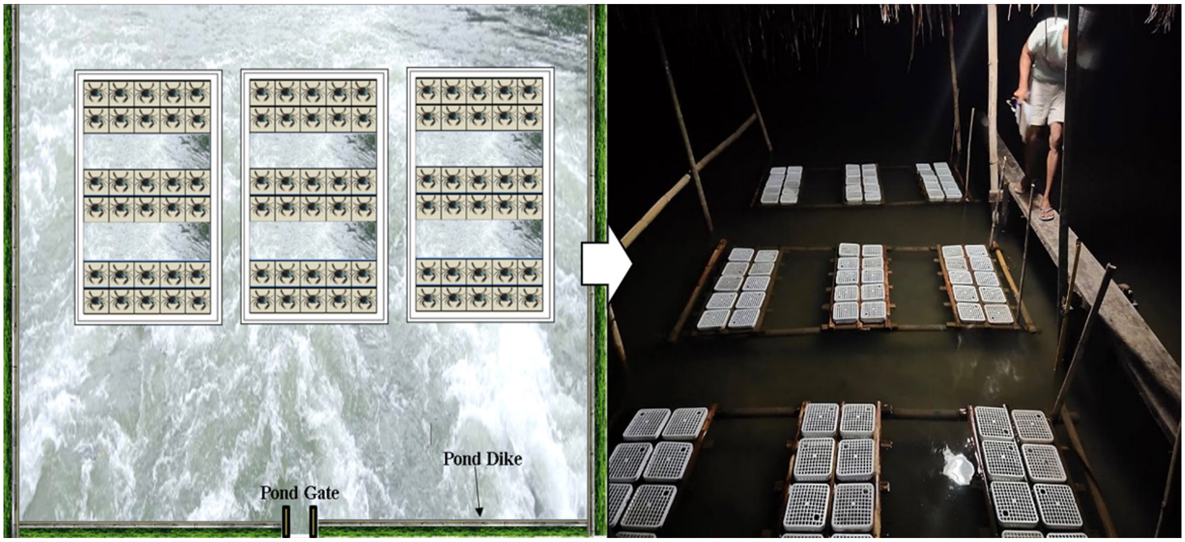

Figure 2. Experimental layout of the study, showing the crab pontoons facing the pond gate (left) and its actual photo during night monitoring (right).

bought directly from two buying stations: Barangay 2, Mercedes and Mambalite, Daet, Camarines Norte. Crab samples were identified morphologically based on the key characters (i.e., frontal lobe spine's shape and height, cheliped carpus and propodus spine, cheliped color, and polygonal patterning on pereiopods) as described by Keenan et al. (1998). Crabs were examined individually to ensure that samples obtained belong to *S. serrata* species using local crabbers' points of identification and by external morphological differentiation following recently published studies (Vincecruz-Abeledo and Lagman 2018; Naim et al. 2020; Asaduzzaman et al. 2021). Fat and healthy crabs were selected during sorting using 4 points of identification: good exoskeleton, live, fat, and complete appendages, as shown in Table 1. The crab farmers, local buyers, and traders adopted this method of selection and grading to ensure quality and high survival during live transport and stocking. Although fat crabs were selected, the degree of fullness of fat was not determined. Fat crabs were selected by gently pressing the underside carapace and abdominal flap. Those with pliant shells and abdomen were considered thin, while those with harder and firmer ones were considered fatter.

Before stocking and taking body measurements, crabs were placed in a plastic basin and gradually sprinkled with pond water. After that, crabs were stocked in individual crab containers, which served as a holding area to give them enough time to adjust to the new environment. Feed was given, and two days after, weak and dead crabs associated with capture stress were removed. Only healthy crabs were selected and stocked in the soft-shelling crab pontoons. This method was done to ensure the high survival of stocks during the culture period.

Crabs were fed with pony fish and telescope snail meat at a 5% feeding rate daily based on the stock's average body weight following Niswar et al. (2017) – recommended feeding on soft-shell crabs. Daily feeding ration was given twice a day: early morning (25%) and late afternoon (75%). Individual feeding was done between 8:00 – 9:00 AM and 5:00 – 6:00 PM. The daily feeding requirement (DFR) was calculated using Tateh AquaBiz mobile application version 2.98.1.

Table 1. Local selection criteria and indications for grading fat and healthy mud crabs used for crab farming.

| Criteria | Indications |
|---|---|
| Healthy | Hard & damaged free shell & frontal lobes; no pierced, cracks & dents on the shell |
| Live | Moving eyestalks, antennae & walking legs |
| Fat | Firm abdomen, hard ventral shell, cheliped & walking legs |
| Complete appendages | With 2 pairs of cheliped, walking & swimming legs |

Daily Feeding Rate as:

$DFR$ = Total stocks (pcs) x SR x BW x FR

where $SR$ is the survival rate (%); $BW$ is the average body weight of stocks (g), and $FR$ is the feeding rate (%).

Pond water management was administered by opening the gate or slab during high tide and closing at low tide to maintain a high water level. Water exchange was done at every high tide to let entry of new water. Water parameters were constantly monitored every hour, such as water temperature, salinity, and dissolved oxygen levels. The gate screen was brushed daily to remove debris that may cause water clogging during pond freshening. Dike leaks and gate slabs were checked occasionally to prevent water loss.

For 57 days, 24-hour monitoring was done to check for water temperature and molting of crabs. Two research assistants were hired to help in the data collection and ensure 24-hour monitoring. Researcher A was assigned in the morning between 9:00 AM–8:00 PM, and Researcher B during the night starting 9:00 PM to 8:00 AM. Primarily, parameters such as temperature were measured, taking notes of the time of sunrise and sunset for diurnal cycles. Water temperature was monitored using a digital salinometer (Smart Sensor Portable Salinity meter AR8012) with built-in automatic temperature compensation (ATC) with a measuring range of 0 – 60°C for the temperature at ± 1°C accuracy. Sampling was done at the front middle of each pontoon, taking notes of time, weather changes, and the diurnal cycle.

The temperature of the water and the incidence of molting (metecdysis) were recorded hourly at the same time. Crab trays were individually checked to record the incidence of molting by towing the crab pontoons under the bridge. All crabs found during hourly monitoring had recently molted, and their exoskeleton is too soft. Lights were used only during monitoring to preserve the dark environment of crabs.

Before untying the crabs' chelipeds, individual body weight and carapace measurements were obtained. The crab's total body weight was measured using a digital weighing scale to the nearest 0.1 g, while a digital vernier caliper was used to measure the carapace length and width to the nearest 0.1 mm. Body measurements were recorded after molting (metecdysis) to determine average growth. Growth increments were calculated by subtracting the stock's pre-molt average body measurement from the post-molt body measurements.

Post-molt (metecdysis) growth in body weight, carapace length, and carapace width were calculated using the following formula:

Weight increment (g) = post-molt weight (g) – pre-molt weight (g)

Carapace length increment (mm) = post-molt carapace length (mm) – pre-molt carapace width (mm)

Carapace width increment (mm) = post-molt carapace width (mm) – pre-molt carapace width (mm)

This study used Microsoft Excel 2016 Office 365 for data analysis**.** Descriptive techniques were used in tabulating data, and calculating frequency, mean, standard deviations, and standard errors. The time of sunrise and sunset was noted using www.timeanddate.com by marking the study location (Mercedes, Bicol, Philippines).

## Post-molt (metecdysis) growth

Table 2 shows the calculated mean of pre-molt, post-molt, and growth increments of crab in weight, carapace length, and width. From the crab's mean pre-molt body measurements of 91.80 g (weight), 53.49 mm (carapace length), and 74.22 mm (carapace width), the recorded mean post-molt body measurements at metecdydis were 126.69 g (weight), 62.02 mm (carapace length), and 85.98 mm (carapace width) with a mean growth increment of 34.89 g in weight, 8.53 mm in carapace length, and 11.76 mm in carapace width.

Table 2 Pre-molt and post-molt mean body measurements, showing the growth increments after molting.

| Measurements | Pre-molt | Post-molt | Increment |
|---|---|---|---|
| Weight (g) | 91.80 | 126.69 | 34.89 |
| Carapace length (mm) | 53.49 | 62.02 | 8.53 |
| Carapace width (mm) | 74.22 | 85.98 | 11.76 |

## Incidence of molting in 24-hour temperature

The recorded water temperature during the culture period varies widely, starting from 26°C to 36°C between 7 May – 2 July 2021 (57 days). Within the recorded 24-hour temperature range, the occurrence of molting was observed between 27°C and 33°C temperature ($\bar{x}$ = 30.0 °C). Overall molting (75.6%) out of the 90 sample stocks varies between these temperature ranges, consisting of: 11.8% under 27°C; 14.7% (28°C), 20.6% (29°C); 20.6% (30°C); 20.6% (31°C); 7.4% (32°C); and 4.4% for 33°C respectively (Figure 3).

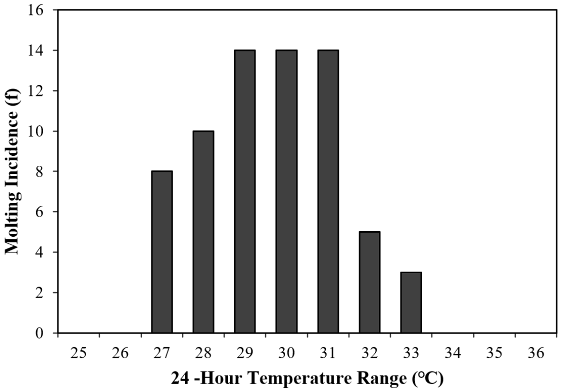

Figure 3. Bar graph showing total molting incidence in the 24-hour temperature range.

## Diurnal cycle of molting

Recorded water temperature within the 57-day culture period greatly varies within 24 hours of monitoring time. The computed weighted mean water temperatures (31.2 ± 1.5°C) during the daytime starting from 7:00 AM to 6:00 PM were observed to be warmer with a 1.9°C temperature difference compared to the nighttime temperatures (29.3 ± 1.2°C) starting from 7:00 PM to 6:00 AM. Temperature trends at nighttime are more stable than daytime temperatures, which fluctuate greatly over time. The daytime temperature ranged from 25.9°C to 36.3°C compared to the nighttime temperatures, which are 25.3°C – 33.8°C. The highest water temperature recorded is in the late afternoon. The diurnal cycle and temperature changes at 12-hour intervals resulted in different molting incidences of 85.29% at nighttime (7:00 PM – 6:00 AM) and 14.7 % at daytime (7:00 AM – 6:00 PM), as shown in Figure 4.

Results showed that external factors that affect molting include water temperature and the diurnal cycle. The optimal water temperature for molting was found to be between 27-31°C, with a high molting incidence of 88.24%. Chaosu and Shaojing (1992) also found that 25-30°C temperatures were optimum for *Scylla serrata* at the zoea stage, where at 35°C, moltings failed, and mortalities happened at 18°C and 20°C. Shelley and Lovatelli (2011) also stated that optimal growth for *S. serrata* was at 30°C, with high growth rates from 25°C to 35°C. Studies showed that temperature is one of the key environmental factors that play a significant role in the life of aquatic organisms (AbolMunafi and Azra 2018; Azra et al. 2020) and exerts a strong influence on the frequency of molting (Bortolin et al. 2011). Hence, one reason why crabs molt at ambient temperatures is that they need to be active to execute water absorption and wriggling out of the old shell. Because low temperatures inhibit the metabolic capacity of mud crabs (Liu et al. 2022), and decrease activity below 20°C (Hill 1980). The active behavior of crabs is observed to be higher at warmer temperatures. Below the $T^{opt}$ no molting was recorded, and beyond the optimal range at higher temperatures between 33-36°C, mortalities and molting failure mostly occur. Molting failure can be observed through a slight opening of the old carapace and an undeveloped new shell inside that resembles a thin membrane.

Furthermore, results supported the statement of Hosamani et al. (2016) that molting is affected by temperature and photoperiod. A related study by Chakraborty (2019) also found that the time of molting (*Scylla spp.*) was during nighttime at 60-70% more than day time. In relation, crabs are known to forage at night for food (Griffen et al. 2012) and with higher activity in the late evening than in mid-morning or mid-day (Mirera and Mtile 2009). The same in soft-shell set-up, crabs' active behavior, and molting occurred at night to prevent predators as they shed their hard shells, which served as their armor, and exposed new and soft bodies, making their pincers weak and helpless to fight against predators. That is why molting frequency is greater when there is a presence of shelter, as Fatihah et al. (2017) stated because the molting process causes the vulnerability of mud crabs to cannibalism or predation (Hamasaki 2003).

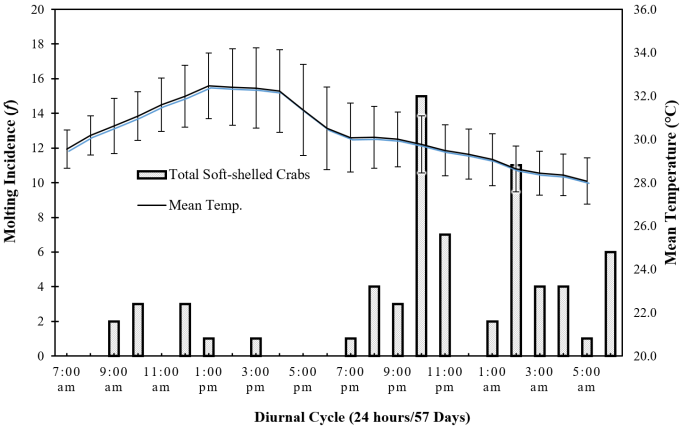

Figure 4. Molting incidence and mean water temperatures at varying diurnal cycles.

To a greater extent, the primary source of variations observed to influence pond water temperature greatly was the heat coming from the sun aside from other external factors like precipitation, tidal cycle, and weather conditions throughout the culture period. With the influence of solar heat energy, water temperature periodically changes between day and night (diurnal cycle). Thus, aside from the dark environment, which favors the nocturnal molting of crabs, it revealed that molting happens at nighttime and early morning because mean water temperatures are within the optimal range ($T_{opt}$), which is ideal for molting.

Understanding the molting of mangrove crabs and their environment can lead to the successful farming of soft-shell crabs. Based on the results and findings, molting happens at nighttime and early morning due to more stable and optimal temperatures associated with the nocturnal behavior of crabs that are naturally active at night and when the environment is dark. Temperature and the diurnal cycle can be used as environmental cues or indicators in monitoring, with an 85% possibility of harvesting newly molted crabs between 7:00 PM and 6:00 AM. Monitoring of molting is advisable at nighttime and early in the morning as higher molting frequencies occur than in the daytime. Indoor soft-shell crab farming is recommended to maintain optimal water temperature and simulate a dark environment.

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## AUTHOR CONTRIBUTIONS

**Biag DC**: Conceptualization, Methodology, Data gathering, Writing-Analysis. **Mendoza Jr. AB**: Conceptualization, Methodology, Reviewing, and Editing.

## CONFLICTS OF INTEREST

The author declares that they have no conflict of interest.

## ETHICS STATEMENT

No animal or human studies were carried out by the authors.

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