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Original Article

Do lunar cycles affect molting of mud crabs Scylla olivacea reared in a closed recirculation system?

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Abstract: Mud crabs grow through molting, a process during which they become highly vulnerable to predation. This makes molting management crucial in crab aquaculture, including practices utilizing crab boxes. Environmental factors such as lunar cycles influence molting in natural habitats. However, research is limited to molting in controlled environments with closed recirculation systems that offer environmental stability, water resource efficiency, and precise feed management. This study investigated the effects of the lunar phase on the molting stages of mud crabs reared in a closed recirculation system. A total of 40 *Scylla olivacea* specimens were reared for three months to observe molting patterns across eight lunar phases: new moon (NM), waxing crescent (WXC), first quarter (FQ), waxing gibbous (WXG), full moon (FM), waning gibbous (WG), last quarter (LQ), and waning crescent (WNC). Overall, 77.5% of crabs were molted during the study period. No molting occurred during the WXC, WXG, WNC, and WG phases. Molting prevalence was higher during FM (22.5±2.5%) and NM (20±2.9%) phases compared to FQ (17.5±1.4%) and LQ (17.5±1.4%). Post-molting weight gain was 31.7±5.9 (NM), 32.2±4.4 (FQ), 31.2±5.9 (FM), and 27±3.2 (LQ), while carapace width (CW) increased by 9.0±1.6 (NM), 8.5±1.3 (FQ), 9.5±2.4 (FM), and 8.2±1.77 mm (LQ), respectively. The results showed molting frequency and growth did not differ significantly between lunar phases NM, FQ, FM and LQ. In closed recirculation aquaculture systems where natural fluctuations such as tidal changes were eliminated, molting remained associated with the lunar cycle, suggesting the persistence of instincts and circadian rhythms in mud crabs in controlled environments. This study provides valuable insights for mud crab farmers, particularly for optimizing productivity by understanding the influence of lunar phases on molting in closed recirculation systems.

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Introduction

Mud crabs (genus *Scylla*) comprise four species with significant potential as aquaculture commodities, particularly in urban aquaculture systems employing closed recirculation technology. As crustaceans, mud crabs grow through a shell replacement process known as molting (Keenan, 1999; Tahya et al., 2016a; Tahya et al., 2017; Sunarti et al., 2020). This critical stage in their life cycle is pivotal for body size increment and post-molting survival (Tahya et al., 2017). During molting, crabs become highly vulnerable to predation and cannibalism. Hence, aquaculture practitioners strive to develop strategies to mitigate cannibalism, minimize mortality, and diversify mud crab products (Tahya et al., 2016b).

Crustaceans are highly influenced by environmental factors that regulate their physiological processes (Engel et al., 2001; Stoner et al., 2010). For instance, temperature, salinity, and natural cycles, including lunar phase progression, can affect molting and reproduction in mud crabs. The gravitational force exerted by the moon alters water conditions in natural habitats. However, in controlled environments, such as recirculating systems, environmental conditions can be tailored to the specific needs of the species (Liu et al., 2022). Studies on crustaceans such as

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Callinectes arcuatus suggest adaptive responses to environmental conditions, including exogenous triggers (DeVries et al., 1983; Achdiat et al., 2024). Although hormonal dynamics often correlate with lunar cycles in crustaceans (Fujaya et al., 2013), further research is needed to understand these effects in controlled environments.

Cultivating crabs in closed recirculation systems offers a promising alternative to minimize environmental stress on crabs and improve control over critical factors such as water quality and cannibalism (Liu et al., 2022). Recirculation systems are typically developed in enclosed spaces, making them suitable for urban aquaculture in areas with limited land availability and controllable environmental conditions. Aquaculture using closed recirculation systems provides opportunities for further research on the influence of lunar phases on the molting process. While several studies have investigated the cultivation of crustacean species in recirculation systems (Lotz and Ogle, 1994; Liu et al., 2022; Yu et al., 2022), research on the effects of lunar cycles in controlled aquaculture environments, such as closed recirculation systems for crab cultivation, remains limited. Therefore, this study aimed to gain insights into the role of lunar phase development in influencing the molting process within a controlled environment, where water capacity serves as a limiting factor, by investigating the impact of lunar phase progression on the molting stages of mud crabs cultured in closed recirculation systems. The study contributes to the scientific foundation for developing more efficient and measured aquaculture strategies, providing insights that can assist crab farmers in optimizing molting timing, adjusting feeding schedules, and managing crab behavior, thereby enhancing productivity and efficiency in mud crab aquaculture.

Materials and Methods

Experimental crabs: This study used 40 healthy individuals of *Scylla olivacea* (Herbst 1796) with body weights ranging between 60-100 g. The crabs were selected from fishermen's catch in Pasangkayu Regency, West Sulawesi Province, Indonesia, ensuring all specimens had intact appendages and were in good health. Prior to the experiment, the crabs were acclimatized in specialized crab-rearing boxes for five days and kept in solitary conditions to minimize stress and reduce the likelihood of autotomy or reflexive voluntary leg detachment. Initial body weight (BW) measurements and carapace width (CW) were made carefully to prevent stress.

Rearing protocol: The rearing experiment was conducted from August to October 2024, using crab boxes arranged vertically in four stacks and horizontally across ten rows. The crab boxes were maintained indoors within a closed recirculation system. Seawater used in the system was salinity-controlled to maintain levels between 27.5-28.0 ppt by dilution with freshwater when evaporation caused salinity to rise. Crab boxes were cleaned every 14 days, and the system was equipped with a skimmer that was cleaned daily to maintain optimal water quality. Fresh fish (*Sardinella* sp.) were provided as feed in the evening at a daily rate of 5% of the crab's body weight.

Observations: Daily observations were conducted at 6:30 AM and 5:00 PM to monitor crab behavior, molting indicators, and correspondence with lunar phases. Post-molt measurements of BW and CW were performed on molted crabs. Data were processed using Microsoft® Excel® for Microsoft 365 (Version 2410) and analyzed using one-way analysis of variance (ANOVA). Water quality parameters, including salinity (measured with an Atago handheld refractometer), dissolved oxygen and temperature (using an Oxygen Meter Lutron DO-5510), and pH (with a digital pH tester) were monitored daily to ensure environmental stability.

Lunar phase progression: The rearing and observation schedule was aligned with the lunar phase calendar, referencing NASA's lunar phase data (https://science.nasa.gov/moon/moon-phases/).

Observations covered phases including new moon (NM), waxing crescent (WXC), first quarter (FQ), waxing gibbous (WXG), full moon (FM), waning gibbous (WG), last quarter (LQ), and waning crescent

Table 1. Lunar calendar in August – October 2024: peak phase date and time of moonrise.

NEW MOON		FIRST QUARTER		FULL MOON		LAST QUARTER	
4 Aug	19:13	12 Aug	23:18	20 Aug	2:25	26 Aug	17:25
3 Sep	9:55	11 Sep	14:05	18 Sep	10:34	25 Sep	2:49
3 Oct	2:49	11 Oct	2:55	17 Oct	19:26	24 Oct	16:03

all times are local time for Palu, Central Sulawesi, Indonesia (https://timeanddate.com/moon/phases)

Table 2. Percentage of crabs molting during each month of the experiment.

	NM	WXC	FQ	WXG	FM	WNG	LQ	WNC
August	10	0	7.5	0	5	0	5	0
September	5		5		10		5	
October	5		5		7.5		7.5	

Table 3. Average and range of BW and CW increments of *Scylla olivacea* in a closed recirculating system

Parameters		**NM**	**FQ**	**FM**	**LQ**
BW Increment (g)	MEAN	31.7±5.9	32.2±4.4	31.2±5.9	27±3.2
	RANGE	24-38	27.8-37.7	20.2-38.2	20.5-30.5
CW Increment (mm)	MEAN	9±1.6	8.5±1.3	9.5±2.4	8.2±1.7
	RANGE	6.3-11.27	7.15-11.03	6.5-14.43	6.07-10.57

(WNC) during August–October 2024. The experiment was designed to eliminate environmental bias by housing crabs in indoor, closed recirculation systems (Table 1).

Results

Molting during lunar phases: The molting of mangrove crabs reared in a recirculation system occurred on specific days surrounding particular lunar phases (Table 2). The molting percentages varied between lunar phases (Fig. 1). Molting percentages were highest during the Full Moon (FM) phase (22.5±2.5%) and New Moon (NM) phase (20±2.9%), followed by the First Quarter (FQ) and Last Quarter (LQ) phases (both 17.5±1.4%). No molting events were recorded during the Waxing Crescent (WXC), Waxing Gibbous (WXG), Waning Gibbous (WNG), and Waning Crescent (WNC) phases. The total percentage of crabs molting over the study period was 77.5%. Analysis of variance (ANOVA) at the 95% confidence level revealed no significant differences in molting percentages between the NM, FQ, FM, and LQ lunar phases over the observation period ($P = 0.75$; $P>0.05$).

Body weight and carapace width increments: The average body weight (BW) increment of molting crabs varied across lunar phases: 31.7±5.9 g during NM, 32.2±4.4 g during FQ, 31.2±5.9 g during FM, and 27±3.2 g during LQ. ANOVA analysis indicated no significant differences in BW increment among lunar phases ($P = 0.24$; $P>0.05$). Similarly, the carapace width (CW) increment recorded was 9±1.6 mm during NM, 8.5±1.3 mm during FQ, 9.5±2.4 mm during FM, and 8.2±1.7 mm during LQ. No significant differences in CW increment were observed across lunar phases ($P = 0.41$; $P>0.05$) (Table 3).

Discussions

Molting in mud crabs is closely linked to external factors influencing physiological processes (Tahya, 2016). This study was conducted in a closed recirculation system, revealing molting patterns correlated with lunar phases. Molting occurred primarily during the new moon, first quarter, full moon, and last quarter phases, while no molting events were observed during other phases such as waxing crescent, waxing gibbous, waning gibbous, and waning crescent. These patterns were documented

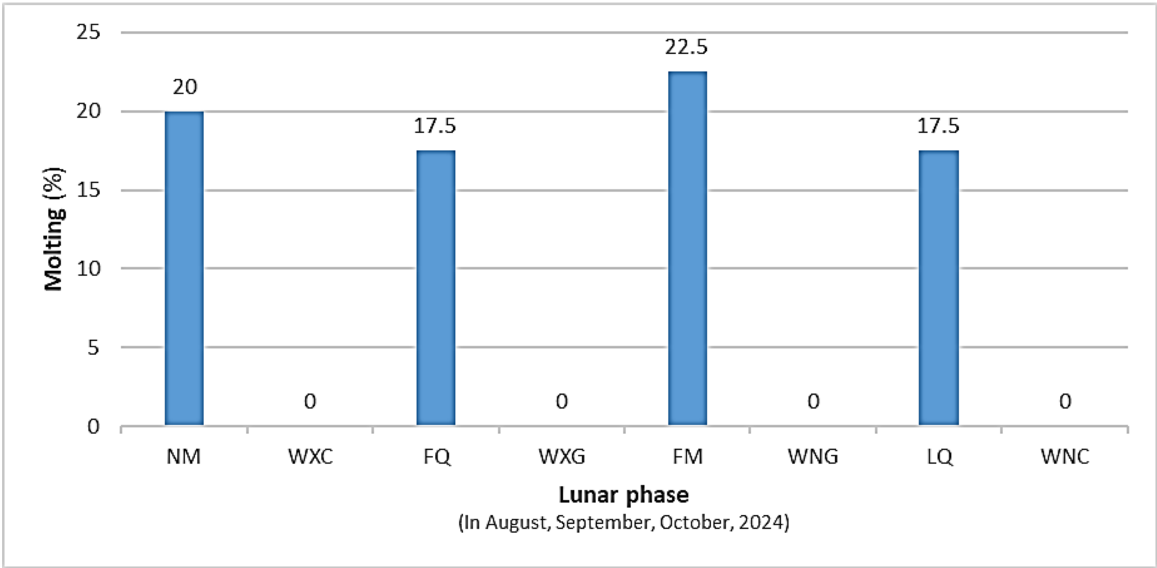

Figure 1. Percentage of crabs molting by lunar phase.

over a three-month lunar cycle. In natural habitats, lunar phase progression affects circadian rhythms, influencing behaviors such as feeding, hiding, and molting (Ferrero et al., 2002; Nishida et al., 2006). Environmental changes like light-dark cycles and tidal fluctuations drive these rhythms. Even under controlled aquaculture conditions, mud crabs appear to maintain their circadian rhythms, suggesting these cycles are an integral aspect of their physiology.

Ecdysis, also known as molting, is a physiological process that occurs throughout the life of mangrove crabs, involving the replacement of the old carapace with a new one, allowing for significant growth (Tahya et al., 2016b). This process is critical in the life cycle of crabs, as the hard carapace restricts further growth. In this study, the growth of crabs reared in a closed recirculation system ranged from 20.2 to 38.2 g in body weight (BW) and 6.3 to 14.43 mm in terms of carapace width (CW). These growth metrics align with results reported for mud crabs cultured in open recirculation systems (Karim et al., 2017; Fujaya et al., 2020). These results indicate successful molting and substantial growth in the closed recirculation system, where environmental factors such as temperature, salinity, and nutrition were carefully managed.

Naturally, the molting process involves several critical stages, culminating in releasing the molting hormone, ecdysone, which triggers the shedding of the exoskeleton. Immediately after molting, crabs absorb water into their bodies along with minerals like calcium to increase their size before the new carapace hardens. This mechanism also occurred in the closed recirculation system used in this study, utilizing resources in the rearing water medium. As a result, molting occurs periodically and can be influenced by circadian factors, environmental conditions, and lunar phase development. This process parallels the molting cycle observed in *Litopenaeus vannamei* shrimp (Lotz and Ogle, 1994) and the hormonal dynamics of molting in crabs cultivated in ponds (Fujaya et al., 2013). Such similarities suggest that the physiological principles governing molting are consistent across species and environmental settings, even in controlled aquaculture systems.

Ecdysteroids are recognized as the primary hormones regulating the molting process in crustaceans, including mangrove crabs (Tahya, 2016; Sunarti et al., 2020). These hormones are produced by the Y-organ located within the crab's carapace and are responsible for triggering various physiological changes necessary for molting preparation (Mykles and Chang, 2020). Ecdysteroids function as biochemical signals that initiate the shell replacement process by stimulating the formation of a new exoskeleton beneath the old shell, which will subsequently be shed. The levels of ecdysteroids in the crab's body increase significantly as the molting phase approaches and decreases after the process. The production and release of ecdysteroids are tightly regulated by Molting Inhibiting Hormone (MIH), which is secreted by the X-organ/sinus gland in the crab's brain. MIH inhibits the activity of the Y-organ in producing ecdysteroids, thus preventing molting (Mykles, 2021). When environmental and internal conditions favor molting, MIH levels decrease, increasing ecdysteroid production. In other words, the balance between MIH and ecdysteroids is critical in determining when molting occurs (Weiner et al., 2021), ensuring that crabs molt only under optimal conditions without encountering nutritional deficiencies, as observed in this study. This study showed an average molting percentage of 20% during the NM phase and 22.5% during the FM phase. Under natural conditions, these lunar phases correspond to higher tidal levels, which may provide natural protection from predators, triggering an instinctual biological response that creates an ideal state for molting. Additionally, the dim moonlight during the new moon phase may offer additional safety for crabs during their vulnerable molting period. This phenomenon indicates an adaptive behavior in crabs to their environment (Stoner et al., 2010), enabling them to utilize natural cycles to optimize survival during molting. Aside from the NM and FM phases, the study also observed crabs undergoing molting during the FQ and LQ phases, with molting percentages of 17.5% for each. However, no molting occurred during the transitional phases between these primary moon phases. The circadian cycle plays a role in regulating the molting process (Ferrero et al., 2002; Chabot et al., 2016), as the production of hormones like ecdysteroids, which control exoskeleton shedding, follows specific daily or lunar patterns. Molting progression may occur more frequently during particular times of the daily cycle, reflecting instinctual habituation similar to the natural molting behavior of mangrove crabs, closely linked to tidal rhythms.

The lunar cycle, consisting of phases such as the new moon, full moon, quarter moon, and transitional periods between these phases, influences various environmental factors, including tidal conditions, nighttime light intensity, and ocean current movements. These environmental changes, driven by lunar gravity, significantly impact the natural habitat of mangrove crabs, particularly in mangrove ecosystems and estuaries. Specific lunar phases, such as around the new moon and full moon, are often associated with higher molting activity in various crustacean species (Ferrero et al., 2002), including mangrove crabs, as observed in this study. Previous research supports these findings, indicating that tidal fluctuations influence crustacean metabolism and hormonal changes, which can trigger molting processes (Ferrero et al., 2002). The physiological phenomena in mangrove crabs are likely closely related to circadian rhythms, as seen in many marine animals inhabiting intertidal zones (Chabot et al., 2016). These rhythms enable the crabs to synchronize their biological processes with the environmental changes in their habitat.

During the study, salinity stability was meticulously controlled daily, allowing the crabs to adapt optimally and minimizing environmental stress (Misbah et al., 2017), which could otherwise disrupt the molting process in a closed recirculation system. For this purpose, salinity was maintained within the range of 27-28 ppt. Maintaining stable salinity enhances the efficiency of new exoskeleton formation, and all the test crabs could undergo the molting process successfully. The combination of controlled environmental factors and the crabs' biological instincts tied to the lunar cycle likely created ideal conditions for patterned and predictable molting. The study's findings that molting occurred during four lunar phases—New Moon (NM), First Quarter (FQ), Full Moon (FM), and Last Quarter (LQ)—indicate a correlation between the lunar cycle and molting timing, even when the crabs were kept in a closed, indoor laboratory environment. External factors like the lunar cycle continued to influence the crabs' biological rhythms, despite the absence of direct environmental variables such as light, currents, and tidal fluctuations in the closed recirculation system.

In closed aquaculture systems like recirculation systems, environmental factors can be controlled more effectively, yet the influence of the lunar cycle remains relevant. While factors such as tidal movements and ocean currents may not play a direct role, changes in light cycles can still impact biological rhythms (Chen et al., 2022). Several studies have observed that simulating lunar light cycles can induce molting patterns similar to natural ones. In closed systems, artificial lighting adjusted to mimic lunar cycles could be a potential strategy to regulate molting timing and enhance the efficiency of crab farming. This approach leverages the crabs' inherent sensitivity to light cues while maintaining the benefits of a controlled environment, offering a promising avenue for optimizing production in closed aquaculture systems.

During the NM, FQ, FM, and LQ phases, the molting frequency was observed to be higher than other phases. Notably, this study recorded no molting events during the WXC, WXG, WNG, and WNC phases. This suggests that specific lunar phases intrinsically influence the physiology of mud crabs through ingrained biological mechanisms such as endogenous cycles or circadian rhythms tied to lunar phases. Moreover, the results showed no significant differences in the molting percentages across the observed lunar phases. These findings support the hypothesis that, despite maintaining tightly controlled environmental factors such as salinity and water quality in the crab box and the closed recirculation system, the lunar cycle continues to play a critical role in regulating hormonal control mechanisms, as previously reported by Fujaya et al. (2013), and aligns with biological rhythms observed in natural settings (Chabot et al., 2016).

The closed recirculation system offers significant advantages in environmental control (Liu et al., 2022); however, this study highlights that biological factors such as the lunar cycle cannot be overlooked. The findings reaffirm that integrating knowledge of the natural rhythms of mud crabs, such as the influence of the lunar cycle, can enhance the success of aquaculture operations within controlled systems. Maintaining optimal environmental conditions in closed recirculation systems, coupled with an understanding of molting patterns, can maximize growth, synchronize molting, and improve aquaculture efficiency. In controlled culture systems where tidal fluctuations are absent, crabs are still capable of undergoing molting. This indicates that while tidal cycles serve as an external trigger, molting is also governed by internal mechanisms, which may include hormonal cycles (Fujaya et al., 2013) and circadian rhythms (Chabot et al., 2016). Within controlled aquaculture systems, other factors such as stable salinity, temperature, and adequate nutrition play a crucial role in supporting the molting process. Although tidal cycles are not present in closed recirculation systems, simulating environmental conditions that mimic natural settings, such as light cycles resembling those in the wild, can help optimize molting processes.

In conclusion, this study demonstrated that molting in mud crabs *Scylla olivacea* occurred predominantly during the new moon (NM), first quarter (FQ), full moon (FM), and last quarter (LQ) phases, with no significant differences in molting frequency across these lunar phases, and no molting occurring during the intermediate moon phases between these four primary moon phases. While molting rates were highest during NM and FM phases, the absence of molting between these phases suggests a strong correlation between lunar cycles and molting behavior. Body weight (BW) and carapace width (CW) increments did not differ significantly among lunar phases, indicating consistent growth across molting events. The findings highlight that, despite the absence of tidal fluctuations in closed recirculation systems, lunar cycles continue to influence the molting process. This suggests that mud crabs retain inherent circadian rhythms and biological instincts even under controlled rearing conditions.

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