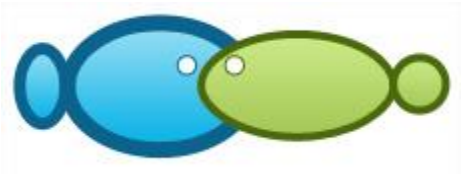

Enhancing pre-mating molting and mating success in immature female mud crabs (*Scylla tranquebarica*) through herbal extract injections

[1]Herlinah Herlinah, [1]Sulaeman Sulaeman, [1]Gunarto Gunarto, [1]Emma Suryati, [1]Andi Tenriulo, [1]Rosmiati Rosmiati, [1]Andi Parenrengi, [1]Samuel Lante, [1]Endang Susianingsih, [2]Early Septiningsih

[1] Research Center for Fishery, National Research and Innovation Agency, Cibinong, Bogor, West Java, Indonesia; [2] Research Center for Conservation of Marine and Inland Water Resources, Cibinong, West Java, Indonesia. Correspondence author: H. Herlinah, herl011@brin.go.id

Abstract. Individual rearing in a cell is an effective rearing technique in preventing cannibalism of mud crab, starting already from the crablet stage. Mud crab, *Scylla tranquebarica*, broodstock reared in a population mixed between male and female to facilitate natural breeding often takes longer (3-4 months) to reach spawned females. The study aimed to look at the performance of herbal extracts against female crab molting. The early inter-molt phase of immature female crabs heading pre-mating molt stage were purchased from the local crab collectors with the following initial data: carapace width (CW) 102.4±1.5 mm, carapace lenght (CL) 67.6±1.1 mm, and body weight (BW) 178.3±6.8 g. The crabs were injected with vitomolt and *Cyperus* sp. rhizome extract before being inserted in a separate crab box and placed at 70 cm depth in an outdoor concrete tank. Simultaneously, three male broodstock weighing >350g were placed individually in a 0.5-ton fiberglass tank in the hatchery building. The newly molted female in the crab-box was immediately transferred to the male tank to mate. The response on growth, molting time, molting frequency, the success on mating and spawning to herbal injection, and the control were recorded. The experiment lasted three months. The results showed that the one dose injection of vitomolt and Cyperus rhizome extract did not cause significant differences in growth, molting time, molting frequency, and survival rate (P>0.05). This research suggested that more extract dosages or injection frequency may be needed to drive *S. tranquebarica* to molt faster.
Key Words: mud crab reproduction, Cyperus rhizome, spawning.

Introduction. The genus of mud crabs, *Scylla* (Brachyura: Portunidae), is found in river estuaries and sheltered coastal habitats which is shallow and estuarine environments (Keenan et al 1998; Sharifian et al 2021). There are four species in this genus found in Indonesia: *Scylla serrata*, *Scylla tranquebarica*, *Scylla olivacea*, and *Scylla paramamosain* also found in Malaysian waters, except for *S. serrata* (Naim et al 2020). Recent studies highlight the significance of comprehending the biology, ecology, and reproductive patterns of the *Scylla* genus, which plays a vital role in selecting the most appropriate species for aquaculture (Naim et al 2020; Bir et al 2020). However, in Indonesia, large-scale cultivation is still in the development stage and mainly focuses on fattening crabs and the production of soft-shell crabs that rely heavily on juveniles from nature.

Mud crab seeding may not have experienced broodstock problems because broodstock can generally spawn daily with a high hatching rate (Azra & Ikhwanuddin 2016). However, broodstock captured from nature are still used (Quinitio et al 2018; Quinitio & Estepa 2011; Shelley & Lovatelli 2011). On the other hand, it is believed that the success of hatchery crabs in consistently producing seeds could only be achieved if broodstock production was sufficiently controlled (Azra & Ikhwanuddin 2016). In addition to the nutritional needs of crab broodstock studied in the last three decades (Azra & Ikhwanuddin 2016), the technique or system of preparing the broodstock is important and determines the success of seed production. The provision of broodstock, which

includes breeding and maturation in the hatchery to get a mature broodstock for the first time, takes a long time that reaches 5 months. The quality of the broodstock that spawns for the first time is usually less good is characterized by a low hatching rate and vitality of larvae (Gunarto et al 2019). Despite the recent studies on seeding, very scarce research was conducted on the provision of broodstock ready to spawn.

Crabs can only perform mating shortly after molting, which is an essential factor in the efforts to provide a controlled broodstock. Molting in crustaceans includes crabs controlled by two hormones, namely a molt-inhibiting hormone and a molting hormone (Allayie et al 2011). Therefore, the acceleration of molting is expected to be accelerated by amplifying the intensity of molting hormones in the crustacean's body or by eliminating molt-inhibiting hormone. The removal of the body's function that produces molt-inhibiting hormone is known as eyestalk ablation (Nakatsuji & Sonobe 2004), while the increased intensity of hormone molting can be accelerated by the treatment of environmental manipulation and hormonal manipulation. The habits of mud crabs migrating to the high seas for spawning may occur because the environmental changes during migration can trigger the hormone molting glands to initiate spawning (Alberts-Hubatsch et al 2016). Research has explored eyestalk ablation for spawning (Allayie et al 2011; Ullah et al 2023), environmental manipulation for molting (Gunarto et al 2020; Sulaeman et al 2021), and hormonal methods for soft crab production (Fujaya 2011; Herlinah et al 2015). However, their use in preparing broodstock for pre-mating molting, mating, and spawning remains unstudied.

In crabs cultivation, molting can either occur naturally or it can be induced through stimulation (Waiho et al 2021) and addition of hormones (Gunamalai et al 2003; Lafont & Dinan 2003; Tamsil & Hasnidar 2018). A possibility of accelerating molting in mud crabs is by injecting molting hormone from herbal ingredients, namely spinach extract (Fujaya 2011; Hasnidar et al 2021). Fujaya (2011) invented a soft-shell crab cultivation technology using vitomolt, consisting of ingredients in the form of spinach plant extract (*Amaranthacea tricolor*) containing 20 hydroxyecdisone (20E).

Another source of molting triggering hormone is mulberry leaves (Herlinah et al 2014; Li et al 2022). Mulberry, *Morus* spp. also known as silk plants because it can be used as a place of life and feed silkworms, *Bombyx mori* (Sanchez 2000). Mulberry contains many chemical compounds such as ecdysterone, inokosterone, lupeol, ß-sitosterol, rutin, moracetin, scopoletin, benzaldehyde, eugenol, linalol, benzyl alcohol, butylamine, acetone, choline, and quercetin (Kim et al 2000). Furthermore, the results of Suryati et al (2021) showed that the extract *Cyperus* sp. rhizomes contained 0.001735% juvenile hormone from dry weight, and that a juvenile hormone application of 100 µg g^{-1} body weight (BW) can respond to the development of vitellogenin and spermatogenic in broodstock candidates of tiger shrimp, to replace the technique of eyestalk ablation. Furthermore, this ingredient can also be tried on candidate mud crab broodstock. The current study investigates the effect of herbal extract injection on the success of pre-mating molt and mating of immature female *S. tranquebarica* reared individually separated from the male.

Material and Method

Broodstock handling. Broodstock female mud crabs in post molt conditions, with a weight of 100-200 g ind^{-1} and the flexible ventral part characteristic for the stadia premating molt (molting for mating), were captured from nature. These specimens were maintained individually in the outdoor tank of 90 tons (90-t) after measuring their size parameters (carapace width=CW, carapace length=CL, and body weight=BW). CW and CL were measured using calipers (Vernier standard) up to 0.1 mm precision, while BW was determined with digital scales with a precision of 0.1 g. Premating molt indicator in female mud crab (*Scylla tranquebarica*) based on abdominal morphology developmental stages are shown in Figure 1. Immature female crabs preparing to molt for mating can be recognized by their rounded U-shaped abdomens (Figure 1 center). On the other hand, those selected for soft crab production are distinguished by their tapering triangular shaped abdomens (Figure 1 left) and mature female indicated by folded, wide

and rounded shaped abdomens with hair along the edges (Figure 1 right). Crabs were fed trash fish, shellfish, and squid (daily intervals depend on the crabs' appetite) 1-2 times a day, as much as 3-5% body weight per day. Water replacement was done every 3-5 days as much as 30-50% of the total volume of water. The molting crabs age and size were recorded, then they were mated and returned to their place for observation of the development of their gonads. Mating was performed in a 1x1x0.5 m^3 rectangular fiberglass tank placed inside the hatchery. Male crab broodstock measuring >300 g were prepared in each mating tank. The duration of the copulation process and the amount at each treatment were recorded. Mating female crabs were immediately disinfected with as much as 150 mg L^{-1} 10% formaldehyde for 3 minutes and then kept in a 90-t trough and separated from male crabs. The mature spawning broodstock were selected and transferred each into a 10 L volume basket floated in a 90-t trough. The incubated crabs were not fed and were transferred into the spawning tank on the seventh day of spawning. Data on fecundity and hatching rate of eggs were calculated, and the maintenance of larvae was carried out according to standard operational procedure of crab seeding (Gunarto et al 2014a; Gunarto et al 2014b; Syafaat et al 2021).

Figure 1. Triangular-shaped abdomen (left), U-shaped abdomen/premating molt stage (center), folded abdomen (right).

Hormone injection. Two types of hormones were studied and the injected specimens were compared to control specimens (without hormone injection). The injection was performed on the mud crab broodstock in the intermoult phase with initial sizes of: CW 102.4±1.5 mm, CL 67.6±1.1 mm, and BW 178.3±6.8 g. Hormone injection was performed on crabs at the beginning of the study, using a 1 mL syringe (26 gauge). Injection treatment with *Cyperus* sp. rhizome extract was compared to commercial vitomolt and control, as follows: A) Vitomolt; B) *Cyperus* sp. rhizome extract; and C) No hormone injection (control). Hormone injection was administered at the crab's swimming legs base before being kept in a crab box. The injection was repeated every week for one month (four times injections). Each treatment was repeated six times. Injected crabs were placed in a crab box suspended on a parallon pipe buoy at about 70 cm (Figure 2). Growth response, molting time or frequency, and survival rate to different herbal treatments were analyzed by the Anova variance test with SPSS-21. The experiment followed a Completely Randomized Design pattern.

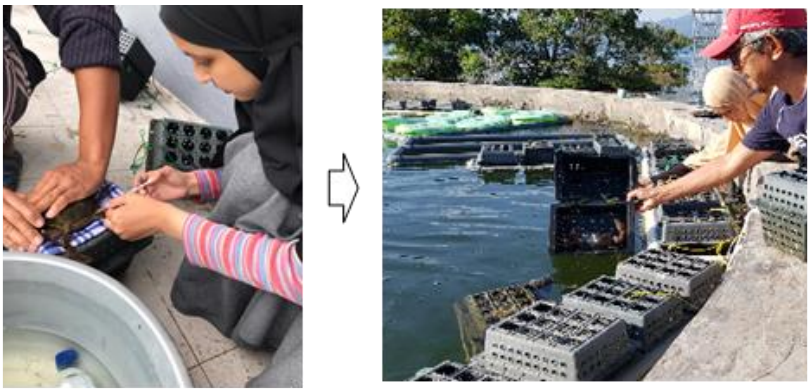

Figure 2. Hormone injection at the base of the crab's swimming legs (left) before being kept in a crab box (right).

Results. After three and a half months of crab maintenance, data collection is presented in Table 1. The hormone injection in this study did not have a significant effect on the growth rate, molting rate, spawning rate, or maturation period of the mud crab broodstock candidates (P>0.05). Additionally, no significant differences were observed in molting frequency or survival rate (SR) across the treatments. While vitomolt has been shown to stimulate molting in smaller crabs (approximately 75 g) (Fujaya et al 2011), it was ineffective in accelerating molting in the larger broodstock candidates (178.3±6.8 g) in this study.

Table 1

The average increment of CL, CW, BW, and days-to-molt duration, molting rate, and the survival rate of broodstock candidate of *Scylla tranquebarica* after hormone injection

Treatment	*CL increment (mm)*	*CW increment (mm)*	*BW increment (g)*	*Days- to-molt (day)*	*Molt rate (%)*	*Days-to-spawn (day)*	*Mature period (day)*	*SR (%)*
Spinach extract (Vitomolt)	8.9± 3.8[a]	14.8± 5.0[a]	57.8± 15.8[a]	60.5± 2.0[a]	62.5	82.3± 2.7[a]	47.3± 2.9[a]	62.5
Cyperus sp. rhizome extract	4.1± 1.3[a]	12.4± 3.1[a]	28.7± 8.0[a]	73.2± 8.2[a]	62.5	75.0± 0.0[a]	31.0± 0[a]	62.5
Control	8.7± 3.3[a]	12.5± 3.0[a]	45.5± 9.5[a]	72.1± 4.5[a]	75	85.0± 5.1[a]	33.7± 4.9[a]	75

CL-carapace length; CW-carapace width; BW-body weight. Different lowercase superscript letters at the same column indicate significant differences among treatments (P<0.05).

Similarly, the treatment using *Cyperus* sp. rhizome extracts did not yield better results than the control across all measured experimental parameters. Factors such as dosage and injection frequency may have influenced the outcome, which could explain the lack of observable effectiveness. Encouragingly, one broodstock from each hormone treatment, observed through the larval maintenance phase, demonstrated strong performance in producing larvae and crablets.

Although the maintenance period for broodstock candidates still lasts about three months (Figure 3), a valuable gained insight is that maintaining broodstock in an individual system within an out-door tank can achieve spawning in a much shorter time (up to three months), compared to in in-door tank system, which may take 4-5 months.

Figure 3. Preparing the broodstock of mud crab, *Scylla tranquebarica*, with individual systems in an out-door concrete tank.

Discussion. The goal of the study was to find out how two different injection types, vitomolt and *Cyperus* sp. rhizome extract, affect growth, molting time, frequency, and mating success in juvenile female crabs. The experiment consisted of injecting herbal extract into juvenile female *S. tranquebarica* to improve pre-mating molting and mating success. The results of this study showed that vitomolt and *Cyperus* sp. rhizome extract

injections had no significant effect on the growth, molting time, molting frequency, or survival rate of crabs compared to the control group. These findings suggest that a single injection dose of the herbal extract used in this experiment may not be sufficient to accelerate molting in brood crabs. Previous research has shown that vitomolt, which contains 20-hydroxyecdysone (20E) of spinach extract, has been effective in increasing molting in smaller crabs (Fujaya 2011). However, in this study, that used larger broodstock crabs (average weight 178.3±6.8 g), the same effectiveness was not found. The results imply that more research is needed to optimize the dosage and frequency of herbal extract injections to effectively stimulate molting in adult mud crab broodstock. Molting is an important process in crustaceans because it can trigger reproductive activities, including mating. Improving the molting process has the potential to shorten the time required for broodstock preparation in an aquaculture setting, thereby increasing the efficiency of seed production. Studies on hormonal manipulation in crustaceans have explored various methods, such as eyestalk ablation, and environmental manipulation to stimulate molting and reproductive activity (Allayie et al 2011; Nakatsuji & Sonobe 2004). While these methods have shown varying degrees of success, herbal extract injections offer a potential alternative that requires further refinement and investigation.

Conclusions. Manipulating hormones by injection of *Cyperus* sp. rhizome extracts and vitomolt did not significantly affect the success of molting and mating of immature female mud crabs in this study, but they provided a basis for further exploration. Optimizing the use of herbal extracts for hormonal manipulation promises to improve the efficiency of mud crab farming, contributing to the sustainable production of seafood and its conservation efforts.

Acknowledgements. The authors would like to acknowledge the Research Institute for Brackishwater Aquaculture and Fisheries Extension (RIBAFE), Maros, for financial support through DIPA-2020. Special thanks also to Nurfadila and Abdul Asis, who kindly provide technical assistance during the laboratory work.

Conflict of interest. The authors declare no conflict of interest.

References

Alberts-Hubatsch H., Lee S. Y., Meynecke J. O., Diele K., Nordhaus I., Wolff M., 2016 Life-history, movement, and habitat use of *Scylla serrata* (Decapoda, Portunidae): current knowledge and future challenges. Hydrobiologia 763(1):5–21.

Allayie S. A., Ravichandran S., Bhat B. A., 2011 Hormonal regulatory role of eyestalk factors on growth of heart in mud crab, *Scylla serrata*. Saudi Journal of Biological Sciences 18(3):283–286.

Azra M. N., Ikhwanuddin M., 2016 A review of maturation diets for mud crab genus Scylla broodstock: present research, problems and future perspective. Saudi Journal of Biological Sciences 23(2):257–267.

Bir J., Saiful I. S., Sabbir W., Rashedul I. M., Islam R., Anisul H. K., 2020 Ecology and reproductive biology of mud crab *Scylla* spp: a study of commercial mud crab in Bangladesh. International Journal of Academic Research and Development 5(2):1–3.

Fujaya Y., 2011 [Growth and molting of mud crabs given different doses of vitomolt]. Jurnal Akuakultur Indonesia 10(1):24-28. [In Indonesian].

Gunamalai V., Kirubagaran R., Subramoniam T., 2003 Sequestration of ecdysteroid hormone into the ovary of the mole crab, *Emerita asiatica* (Milne Edwards). Current Science 85(4):493–496.

Gunarto G., Sulaeman S., Herlinah H., 2019 The preference size of male mud crab, *Scylla tranquebarica* at success mating with female, *Scylla olivacea* in controlled tanks. Aquacultura Indonesiana 20(2):37-45.

Gunarto G., Tampangallo B. R., Herlinah H., Syafaat M. N., 2014a [Maintenance of mud crab larvae, *Scylla serrata* with natural feeding of different types of enrichment]. Proceeding of Forum Inovasi Teknologi Akuakultur 2014:653-665. [In Indonesian].
Gunarto, Herlinah, Parenrengi A., 2014b [Technical instruction for mud crab hatchery, *Scylla* spp]. Balai Penelitian dan Pengembangan Budidaya Air Payau, 34 p. [In Indonesian].
Hasnidar H., Tamsil A., Wamnebo M. I., 2021 The effects of the amaranth extract (*Amaranthus* spp.) on the molting of orange mud crab (*Scylla olivacea*). AACL Bioflux 14(2):1036–1045.
Herlinah H., Tenriulo A., Suryati E., 2014 [The hormone ecdysterone from mulberry leaf extract, *Morus* spp. as a moulting stimulant in mud crabs]. Journal Riset Akuakultur 9(3):387-397. [In Indonesian].
Keenan C. P., Davie P. J. F., Mann D. L., 1998 A revision of the genus Scylla de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). Raffles Bulletin of Zoology 46(1):217–245.
Kim S. Y., Gao J. J., Kang H. K., 2000 Two flavonoids from the leaves of *Morus alba* induce differentiation of the human promyelocytic leukemia (L-60) cell line. Biology Pharmaceutical Bulletin 23(4):451–455.
Lafont R., Dinan L., 2003 Practical uses for ecdysteroids in mammals including humans: and update. Journal of Insect Science 3(7):1–30.
Li R., Su X., Zhou R., Zhang Y., Wang T., 2022 Molecular mechanism of mulberry response to drought stress reveale dby complementary transcriptomic and iTRAQ analyses. BMC Plant Biology 22(1):1–18.
Manuel D. S., 2000 World distribution and utilization of mulberry, potential for animal feeding. FAO Electronic Conference on Mulberry for Animal Production, 11 p.
Naim D. M., Nor S. A. M., Mahboob S., 2020 Reassessment of species distribution and occurrence of mud crab (*Scylla* spp., Portunidae) in Malaysia through morphological and molecular identification. Saudi Journal of Biological Sciences 27(2):643–652.
Nakatsuji T., Sonobe H., 2004 Regulation of ecdysteroid secretion from the Y-organ by molt-inhibiting hormone in the American crayfish, *Procambarus clarkii*. General and Comparative Endocrinology 135(3):358–364.
Quinitio E. T., dela Cruz-Huervana J. J., Parado-Estepa F. D., 2018 Quality assessment of newly hatched mud crab *Scylla serrata* larvae. Aquaculture Research 49(1):75–80.
Quinitio E. T., Estepa F. D. P., 2011 Survival and growth of Mud crab, *Scylla serrata*, juveniles subjected to removal or trimming of chelipeds. Aquaculture 318(1–2):229–234.
Sharifian S., Kamrani E., Saeedi H., 2021 Global future distributions of mangrove crabs in response to climate change. Wetlands 41(99):1-14.
Shelley C. C., Lovatelli A., 2011 Mud crab aquaculture - a practical manual AO Fisheries and Aquaculture Technical Paper. No. 567, 78 p.
Suryati E., Lante S., Tenriulo A., Rosmiati, Parenrengi A., 2021 Potential of methyl fernesoate isolate from the Cyperus extract in prospective broodstock of shrimp (*Penaeus monodon* Fab). IOP Conference Series: Earth and Environmental Science 860:1-11.
Syafaat M. N., Azra M. N., Waiho K., Fazhan H., Abol-Munafi A. B., Ishak S. D., Syahnon M., Ghazali A., Ma H., Ikhwanuddin M., 2021 A review of the nursery culture of mud crabs, genus Scylla: current progress and future directions. Animals 11:1-15.
Tamsil A., Hasnidar 2018 The effect of molting hormone (20-hydroxyecdyson) on molting of mud crab (*Scylla olivacea* Herbst, 1976). Ecology, Environment and Conservation 24(2):960–967.
Waiho K., Ikhwanuddin M., Baylon J. C., Jalilah M., Rukminasari N., Fujaya Y., Fazhan H., 2021 Moult induction methods in soft-shell crab production. Aquaculture Research 52(9):4026–4042.

Received: 15 July 2024. Accepted: 27 December 2024. Published online: 19 January 2025.
Authors:
Herlinah Herlinah, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, Bogor, West Java, Indonesia, e-mail: herl011@brin.go.id
Sulaeman Sulaeman, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, Bogor, West Java, e-mail: sulaeman.marhali@gmail.com
Gunarto Gunarto, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, West Java, Indonesia, e-mail: guna019@brin.go.id
Emma Suryati, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, West Java, Indonesia, e-mail: emma005@brin.go.id
Andi Tenriulo, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, West Java, Indonesia, e-mail: andi054@brin.go.id
Rosmiati Rosmiati, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, West Java, Indonesia, e-mail: rosm005@brin.go.id
Andi Parenrengi, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, West Java, Indonesia, e-mail: andi053@brin.go.id
Samuel Lante, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, West Java, Indonesia, e-mail: samu005@brin.go.id
Endang Susianingsih, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, West Java, Indonesia, e-mail: enda055@brin.go.id
Early Septiningsih, Research Center for Fishery, National Research and Innovation Agency, Cibinong 16911, West Java, Indonesia, e-mail: earl002@brin.go.id

How to cite this article:
Herlinah H., Sulaeman S., Gunarto G., Suryati E., Tenriulo A., Rosmiati R., Parenrengi A., Lante S., Susianingsih E., Septiningsih E., 2025 Enhancing pre-mating molting and mating success in immature female mud crabs (*Scylla tranquebarica*) through herbal extract injections. AACL Bioflux 18(1):119-125.