Grouper: Jurnal Ilmiah Perikanan
Vol. 17 No. 2 (2026) pp. 561 - 569
pISSN: 2086-8480 | eISSN: 2716-2702
Journal Homepage: https://grouper.unisla.ac.id/index.php/grouper

Preliminary Observation of a Recirculating Aquaculture System for Producing Berried Female Mud Crab (*Scylla paramamosain*)

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Submitted: 26 August 2026 Revised: 25 September 2026 Accepted: 30 September 2026

Keywords:
Aquaculture; Blue Economy; Broodstock; Crustacean; Reproduction

ABSTRACT

Mud crab aquaculture relies heavily on wild broodstock, while the availability of hatchery-produced seed remains a major constraint to its expansion. This preliminary study explored the feasibility of maintaining and inducing spawning of female *Scylla paramamosain* broodstock in a recirculating aquaculture system without eyestalk ablation. Five female crabs with mature ovaries at stages III–IV were individually maintained in the system and monitored for survival, development to the berried stage, egg extrusion, and hatching. Two of the five females (40%) spawned and developed into berried females, with egg extrusion occurring on Days 35 and 37 after stocking. One of the two berried females survived to hatching on Day 43, corresponding to 50% of berried females successfully reaching hatching stage. Both females that reached the berried stage were initially classified as having stage IV ovaries, whereas none of the stage III females reached the berried stage. Water quality remained relatively stable throughout the culture period, with temperature, salinity, pH, dissolved oxygen, and ammonia maintained within suitable ranges for mud crab culture. These observations provide preliminary evidence that a recirculating aquaculture system may support reproductive progression of *Scylla paramamosain* broodstock without eyestalk ablation. However, the small sample size and absence of a control treatment limit definitive conclusions regarding system effectiveness. Further studies with larger sample sizes and appropriate controls are needed to clarify the factors affecting reproductive success.

INTRODUCTION

Mud crabs of the genus *Scylla* are commercially important portunid crabs widely distributed in tropical and subtropical coastal environments, particularly throughout the Indo-Pacific region. Their high market value, desirable meat quality, and strong consumer demand have established mud crabs as an important resource for fisheries and aquaculture (Alberts-Hubatsch *et al.,* 2016; Hidir *et al.,* 2022; Shelley & Lovatelli, 2011). The commercial importance of crab resources is also reflected in increasing demand from major crab-importing countries, where annual crab imports reportedly increased from approximately 260,000 to 290,000 tonnes (Muhlis *et al.,* 2021). Despite Indonesia's considerable potential for brackishwater aquaculture, mud crab production has remained strongly dependent on capture fisheries. During 2015–2017, approximately 75–85% of production originated from capture fisheries, whereas aquaculture contributed only 15–25% (Andayani, 2022). Continued reliance on wild populations for production and aquaculture inputs may increase pressure on natural stocks and raise concerns regarding the long-term sustainability of mud crab resources (Quinitio *et al.,* 2011).

In response to exploitation pressure, the Indonesian government has strengthened regulations to support the sustainable utilization and conservation of mud crab resources. Under Minister of Maritime Affairs and Fisheries Regulation No. 7 of 2024, mud crabs (*Scylla* spp.) harvested for consumption must not be in a berried condition and must have a carapace width exceeding 12 cm or a body weight exceeding 150 g per individual. These measures are intended to protect natural stocks and reproductive potential, thereby supporting the long-term sustainability of mud crab resources. In parallel, strengthening hatchery-based seed production can provide a complementary approach to reduce dependence on wild populations while supporting continued aquaculture development. However, inadequate seed supply remains a major constraint for mud crab farming, while the high quality and survival of hatchery-produced larvae remain critical challenges for achieving consistent mass seed production (Liew *et al.,* 2024). Therefore, improving hatchery production requires effective management throughout the production cycle, beginning with broodstock management, which provides the foundation for subsequent egg incubation, hatching, larval rearing, and seed production.

To address the need for more controllable and welfare-oriented broodstock management, recirculating aquaculture systems (RAS) have emerged as a promising approach for mud crab culture. Compared with conventional earthen ponds, RAS allows greater control over water quality, temperature, and biosecurity, providing a more stable environment for captive broodstock management (Murak *et al.,* 2026; Van Calunod *et al.,* 2024). Recent studies have also indicated the potential of recirculating systems to support the production of gravid female mud crabs without eyestalk ablation (Hajisamae *et al.,* 2025). These findings suggest that RAS may offer a suitable platform for exploring alternative broodstock management strategies while reducing reliance on invasive reproductive techniques.

Building on these findings, this preliminary study explored the feasibility of maintaining *Scylla paramamosain* broodstock in a RAS without eyestalk ablation. The study focused on observing preliminary reproductive outcomes, including the occurrence of the berried stage, egg extrusion, and hatching. The findings are intended to provide initial evidence of the potential of RAS as a controllable and welfare-oriented approach for mud crab broodstock management and to inform further studies under more rigorous experimental conditions.

METHOD

Collection, Selection, and Acclimation of Crab Broodstock

Female mud crab (*Scylla paramamosain*) broodstock were collected from natural habitats in Tanjungsari, Kupang, Jabon District, Sidoarjo Regency, East Java. They were then transported to the semi-outdoor laboratory of the Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia. Broodstock with mature ovaries at stages III–IV were selected by assessing ovarian development via external visual inspection using a light-emitting diode (LED) flashlight. At these stages, the ovaries typically extend to the margins of the carapace and occupy approximately 75-100% of the internal body cavity, following the methodology outlined by Chema *et al.* (2024). Upon arrival at the laboratory, the crabs were cleaned and disinfected by immersing them in a 150 mg L^{-1} formaldehyde solution for 30 minutes, followed by a 3-day acclimation period (Baylon, 2011; Waiho *et al.,* 2015; Fazhan *et al.,* 2022.). Before stocking, the initial carapace width and body weight of each individual broodstock were measured using a vernier caliper and a digital balance accurate to two decimal places. Subsequently, five female crabs were stocked separately in a recirculating aquaculture system (RAS), with one crab per rearing tank. The experiment was conducted over a period of 6 weeks, continuing until the hatching of the last female crab or until total mortality occurred.

Recirculating Aquaculture System (RAS) Setup

The RAS was composed of five cylindrical plastic rearing tanks, each with a 30 L capacity. To simulate the natural benthic environment of mud crabs, a sand substrate

approximately 5 cm thick was provided in each tank (Chema *et al.,* 2024; Fazhan *et al.,* 2022). The water volume was maintained at 28 L per tank, yielding a combined total volume of 140 L across the rearing units. Water overflowing from the rearing tanks flowed through 1-inch overflow pipes into a filter sump tank. This sump was modified from a 69 L Styrofoam box (59 × 45 × 26 cm), with an actual working water volume of approximately 50 L. Water treatment comprised mechanical filtration using coarse filter wool, followed by biological filtration utilizing crushed coral as the biofilter medium (Estim & Mustafa, 2010; Subagja *et al.,* 2025). The treated effluent was then continuously recirculated back to the rearing tanks via a submersible pump operating at a manufacturer rated flow rate of 800 L h^{-1}.

Water Quality Management

The water quality parameters in the rearing system were maintained at a salinity of 30-35 ppt, alkalinity ≥120 mg L^{-1}, temperature around 28 °C, pH 7.8-8.2, and dissolved oxygen (DO) >5 mg L^{-1} (Chema *et al.,* 2024; Ikhwanuddin *et al.,* 2015). Water quality parameters, including temperature, salinity, pH, DO, and ammonia, were monitored on a weekly basis following the water exchange. Throughout the trial, a weekly water exchange of approximately 20% was performed, calculated from the 50 L working volume of the filter sump.

Diet and Feeding Regime

The broodstock were fed with blood cockles at a daily ration of 3–5% of total body weight (Chema *et al.,* 2024; Herlinah & Septiningsih, 2015), dispensed in a single daily meal at 16:00. Prior to feeding, the cockles were cleaned and frozen for preservation. Unconsumed feed was systematically removed from the rearing units on the subsequent day.

Data Collection and Calculations

The experimental animals were continuously observed throughout the study duration to evaluate survival rates, feeding behavioral responses, egg extrusion, and hatching performance. Mortality instances and critical milestones were logged chronologically. A female was classified as attaining the berried stage once oocytes were successfully extruded and secured onto the abdominal pleopods.

Percentage of females reaching the berried stage

The percentage of females reaching the berried stage was calculated as:

$$\text{Berried female rate (\%)} = \frac{\text{Number of females reaching the berried stage}}{\text{Total number of females stocked}} \text{x}100$$

Hatching success

Hatching success was determined according to Wu *et al.* (2010) and calculated as:

$$\text{Hatching success (\%)} = \frac{\text{Number of berried females successfully hatched}}{\text{Total number of surviving berried females}} \text{ x } 100$$

Latency period to egg extrusion

The latency period to egg extrusion was calculated according to Varadarajan and Subramonian (1982) as:

$$\text{Latency period (days)} = \text{Date of egg extrusion} - \text{Stocking date}$$

Data Analysis

Because this study was designed as a preliminary assessment utilizing a limited broodstock size, reproductive outputs and survival rates were evaluated using descriptive statistics. Key reproductive performance indicators included the percentage of females reaching the berried stage, hatching success rate, and the latency period before egg extrusion.

RESULT AND DISCUSSION

This study provides a preliminary observation on the application of a Recirculating Aquaculture System (RAS) to manage mud crab (*Scylla paramamosain*) broodstock at ovarian stages III–IV without eyestalk ablation. The study found that 40% of the broodstock (2 out of 5 females) successfully developed into berried females, undergoing egg extrusion on days 35 and 37 after the start of the experiment, respectively (Table 1).

Table 1. Morphological characteristics, initial ovarian stages, and reproductive performance of female mud crab (*Scylla paramamosain*) broodstocks reared in the recirculating aquaculture system (RAS).

Broodstock ID	Body Weight (g)	Carapace Width, CW (cm)	Carapace Length, CL (cm)	Initial Ovarian Stage	Latency Period (days)	Berried Stage Reached
Broodstock 1	340	15.63	9.29	Stage III	—*	No
Broodstock 2	300	12.26	6.51	Stage IV	35	Yes
Broodstock 3	310	12.08	6.55	Stage IV	37	Yes
Broodstock 4	240	11.54	6.19	Stage III	—*	No
Broodstock 5	200	10.49	5.54	Stage IV	—*	No
Mean ± SD / Summary	278.0 ± 56.7	12.40 ± 1.94	6.82 ± 1.44	—	36.0 ± 1.41**	40.0%

Note: CW = Carapace width; CL = Carapace length.
* Not applicable as these individuals failed to extrude eggs during the experimental period.
** Calculated from females that successfully extruded eggs (n = 2).
*** Overall berried female rate = (number of berried females / total stocked females) × 100.

These findings demonstrate that under the present experimental conditions, the RAS has the potential to support reproductive processes in physiologically mature broodstock. Nevertheless, due to the absence of a non-RAS control group, it cannot be conclusively determined that the RAS directly caused the progression to the berried female stage.

The 40% berried female rate can be contextually compared with the study by Yu *et al.* (2026) *on S. paramamosain* reared in RAS who revealed that eyestalk ablation, rather than temperature manipulation alone, was the primary driver for significantly increasing spawning rates. This outcome implies that RAS environments must be coupled with specific physiological interventions or external stimuli to effectively induce reproduction in female broodstock. Additionally, according to Zhou *et al.* (2026), mated *S. paramamosain* maintained in a RAS achieved higher stages of ovarian maturation relative to unmated females. This suggests that mating factors and associated physiological signals may be important for ovarian development and reproduction. Therefore, the RAS should be viewed as a supportive environment for reproductive processes rather than an isolated spawning trigger. These results provide intriguing preliminary evidence that broodstock with developed ovaries can successfully transition to the berried stage within a RAS, entirely without eyestalk ablation. This is in agreement with Hajisamae *et al.* (2022) who validated the feasibility of RAS-based berried female production without eyestalk ablation. Specifically, this approach has achieved an egg extrusion success rate of 70–80% within 30 days of stocking.

Regarding initial ovarian development, the two broodstocks that successfully extruded eggs possessed stage IV ovaries, whereas the stage III females failed to reach the berried stage. However, these data should not be interpreted as causation between stage IV ovaries and spawning success, primarily due to the small sample size per group and because evaluating the impact of specific ovarian stages was beyond the scope of this study's design. Therefore, the observed results are merely a preliminary trend indicating that female crabs at stage IV before the experiment may be more physiologically ready for spawning than stage III crabs. This is consistent with mud crab broodstock management principles, which state that ovarian maturity before entering the reproductive phase is a crucial factor for spawning success (Azra & Ikhwanuddin, 2016). Furthermore, the broodstock selection protocol employed in this study

aligns with practices reported from Thailand for *S. paramamosain*. Hajisamae *et al.* (2025) noted that broodstock production entails selecting healthy females weighing approximately 200–400 g with advanced ovarian development, specifically stage IV, prior to stocking into the production system. However, such information should be interpreted as localized management insights derived from Thai broodstock production systems, rather than as a definitive global standard or mandatory benchmark for all broodstock culture operations.

The duration from initial RAS stocking to egg extrusion was 35 and 37 days for the two successful broodstock, respectively. The negligible difference of only 2 days suggests that both individuals exhibited highly comparable developmental timelines leading to spawning. Even though continuous monitoring of ovarian development and related physiological indicators of maturation were not performed, the 35–37day duration should be interpreted merely as the observed latency period under the present experimental conditions, rather than as a definitive standard for *S. paramamosain*. Past research indicates that the timeframe from broodstock conditioning to spawning can be affected by the induction technique and culture environment. Notably, Djunaidah *et al.* (2003) reported no significant differences in the duration from eyestalk ablation to spawning across various feeding treatments. On the other hand, Zeng (2007) indicated that combining eyestalk ablation with thermal control successfully promotes out of season spawning. Thus, the latency timeframe recorded here is likely the synergistic result of initial broodstock maturity, rearing conditions, and ongoing reproductive progression prior to entering the RAS facility. When compared with the findings of Hajisamae *et al.* (2022) who achieved a 70–80% egg extrusion success rate within 30 days of stocking the results here showed egg extrusion at days 35 and 37, indicating a marginally extended duration. Even so, this period aligns with the general 20– 40day duration required for broodstock maintenance in spawning tanks as outlined in Thai management frameworks (Hajisamae *et al.,* 2025). These differences in duration and success rate may be related to variations in sample size, initial conditions of the broodstock, feeding regimes, system size and configuration, as well as specific environmental management details. Consequently, the findings of this study should be viewed as preliminary evidence supporting the potential of integrating a RAS with the selection of stage III–IV broodstock for producing berried females without eyestalk ablation, rather than concluding that the present system matches or surpasses the efficiency of previously documented systems.Water quality remained relatively stable throughout the experimental period, with temperature ranging from 27.4–28.7 °C, salinity from 30–32 ppt, pH from 7.50–7.84, dissolved oxygen (DO) from 5.17–5.52 mg/L, and ammonia remaining undetectable. Temperature is an important environmental factor affecting several biological processes in mud crabs, including reproduction, spawning, embryonic development, feeding, growth, and survival (Pedapoli & Ramudu, 2014; Ruscoe *et al.,* 2004). The temperature recorded in the present study was within the range commonly used for mud crab broodstock culture (25–32 °C; FAO, 2011) and was comparable to conditions reported for *S. paramamosain* broodstock undergoing egg extrusion, which were maintained at 28–31 °C (Li *et al.,* 2022). In addition, the observed pH and DO levels were broadly comparable to those reported in previous *S. paramamosain* studies (pH 7.9–8.2 and DO 4–5 mg/L; Li *et al.,* 2022). The relatively stable water quality, particularly temperature and DO, may therefore have provided suitable environmental conditions for broodstock maintenance and reproductive progression. However, because this study did not include a control treatment or manipulate individual water-quality variables, their specific effects on egg extrusion cannot be determined. The absence of detectable ammonia may also have helped minimize environmental stress during broodstock culture.

Table 2. Weekly water quality parameters recorded in the recirculating aquaculture system (RAS) during the broodstock culture period.

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Temperature	27.4 oC	27.9 oC	27.7 oC	28.1 oC	28.7 oC	28.5 oC
DO	5.52 mg/L	5.25 mg/L	5.43 mg/L	5.33 mg/L	5.20 mg/L	5.17 mg/L
Salinity	30 ppt	30 ppt	32 ppt	30 ppt	32 ppt	32 ppt
pH	7.84	7.75	7.76	7.67	7.5	7.5

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Ammonia	ND*	ND*	ND*	ND*	ND*	ND*

Besides water quality, the sand substrate in each tank may have influenced behavioral patterns and readiness for spawning. The use of a ~5 cm sand layer in this study likely facilitated natural behavioral expressions in the broodstock, mimicking their natural habitat. Fazhan *et al.* (2022) found that the use of sand can promote egg extrusion in mud crabs of the genus *Scylla*, and that fine grained sand yields better results than coarse sand and the absence of sand. However, because sediment grain sizes were not quantified in the present trial, the substrate should not be designated as fine sand, and no definitive link can be established between particle size and spawning induction. Rather, these results collectively suggest that integrating a sandy bottom into the rearing setup warrants further investigation as a potential factor for improving broodstock management protocols.

Nutritional and feeding dynamics should also be evaluated alongside the experimental outcomes, as the broodstock exhibited a relatively limited feeding response during the 3-day acclimation period and the initial 3–4 days post-stocking in the RAS. This early decrease in feed intake likely reflects stress induced by capture, transportation, and environmental acclimatization. However, because this study did not measure actual feed intake, remaining feed weight, energy expenditure, or biochemical composition changes such as lipids and glycogen, it cannot be determined that the decreased feed intake was the direct cause of mortality or the failure to develop into berried females. The review by Azra and Ikhwanuddin (2016) noted that *Scylla* broodstock exhibit high nutritional demands associated with vitellogenesis, specifically requiring substantial amounts of proteins, lipids, fatty acids, and amino acids. Meanwhile, Djunaidah *et al.* (2003) demonstrated that feed quality and type are critical elements that must be considered in broodstock management. Thus, the early reduction in feed intake represents a parameter that should be tracked in future investigations, emphasizing the systematic quantification of dietary intake and maternal physiological energy reserves.

The mortality of broodstock during the experiment also represents a significant limitation of this study, as three female crabs failed to survive until the berried stage, with death occurring at varying intervals from days 2 to 21 of the experiment. This mortality could be attributed to multiple factors, including capture and transport stress, acclimation challenges, variations in the initial physiological state of the broodstock, or energetic constraints. Nevertheless, since the present study did not gather pathological, physiological, or energy status data, the definitive cause of death remains unidentified.

The study by Nemeño *et al.* (2026) provides an interesting context to this issue. Using a RAS with a polychaete-assisted biofilter and isolating individual female *Scylla* serrata resulted in a survival rate of 70.37 ± 6.41% and a spawning rate of 61.11 ± 4.80%. This was higher than in non-RAS systems, which had a survival rate of 51.85 ± 5.78% and a spawning rate of 35.18 ± 10.14%, respectively. The researchers suggested that individual housing may help reduce cannibalism, and the stable environment within the RAS may contribute to broodstock performance. However, in this study, the female crabs were housed individually from the beginning, with one crab per tank. Therefore, cannibalism is unlikely to be the main explanation for the observed mortality. The differences in survival rates should be considered along with differences in crab species, sample size, system volume, feeding regimes, experimental duration, and biofilter composition between the two studies.

Regarding hatching success, out of the two female crabs that achieved egg extrusion, only one survived until the eggs hatched, while the other died on the same day as egg extrusion. Consequently, when calculated at the broodstock level, 50% of the berried females successfully hatched their eggs. These findings should be explicitly defined as the 'proportion of berried females that successfully achieved hatching,' rather than employing the term 'egg hatching rate,' which typically denotes the percentage of individual eggs hatching into larvae. This

clarification is essential since the present study did not quantify the total egg count or the individual hatching success per egg.

By comparison, Nemeño *et al.* (2026) documented hatching success rates of 55.55 ± 4.81% and 20.37 ± 8.01% for RAS and non-RAS configurations, respectively. Crucially, however, their metric for hatching success was formulated based on the initial number of stocked females that eventually produced larvae, whereas the 50% proportion reported herein was computed strictly from the cohort of berried females. Thus, despite the apparent numerical similarities, direct comparisons should be avoided when inferring relative system efficacies.

CONCLUSION

Under the conditions tested, the recirculating aquaculture system (RAS) provided a supportive rearing environment for *Scylla paramamosain* broodstock, with 2 of 5 females (40%) developing into berried females without eyestalk ablation. Egg extrusion occurred on Days 35 and 37, while one berried female successfully hatched its egg clutch on Day 43, corresponding to 50% of berried females successfully reaching hatching. Both females that reached the berried stage were initially classified as ovarian stage IV, whereas none of the stage III females successfully reached the berried stage. However, the small sample size and absence of a control treatment prevent definitive conclusions regarding the effectiveness of RAS or the influence of initial ovarian stage on reproductive success.

Water quality remained relatively stable throughout the experimental period, with temperature, salinity, pH, dissolved oxygen, and ammonia maintained within ranges broadly comparable to those reported for *S. paramamosain* culture and embryonic development. The findings therefore suggest that RAS has potential as a broodstock management approach for supporting reproductive progression in *S. paramamosain*, but its contribution to egg extrusion and hatching requires further evaluation under controlled experimental conditions. Future studies should employ larger sample sizes and appropriate control treatments while systematically examining broodstock condition, initial ovarian stage, feeding and energy status, environmental parameters, and reproductive performance to clarify the factors governing spawning and hatching success in RAS-based broodstock culture.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Universitas Airlangga, particularly the Faculty of Fisheries and Marine Sciences (MERR C Campus), for providing the facilities, financial support, and mud crab broodstock necessary for this research. The authors also acknowledge the Faculty of Science and Technology, Prince of Songkla University, Thailand, and the PSU-TUYF Charitable Trust for the financial support of the first author's M.Sc. candidature. Furthermore, the authors gratefully acknowledge the master's students and staff of the Faculty of Fisheries and Marine Sciences for their invaluable assistance and encouragement throughout this study.

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