Fisheries Journal, 16 (4), 2052-2060 (2026)
http://doi.org/10.29303/jp.v16i4.2226

EFFECT OF *Rhizophora mucronata* LEAF EXTRACT ON THE GONAD MATURITY OF MUD CRAB *Scylla olivacea*

Pengaruh Ekstrak Daun *Rhizophora mucronata* Terhadap Kematangan Gonad Kepiting *Scylla olivacea*

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(Received June 30th 2026; Accepted August 17th 2026)

ABSTRACT

Female mud crabs are a high-value fishery commodity due to the market demand for their gonadal development potential. To date, market supply remains predominantly dependent on wild-caught populations. This condition threatens the sustainability of mud crab stocks, both for market supply and the conservation of natural populations. The present study aimed to evaluate the effect of mangrove leaf extract (*Rhizophora mucronata*) on the gonadal maturation of mud crab (*Scylla olivacea*). The study employed a Completely Randomized Design, consisting of four dosages of mangrove leaf extract (0, 0.5, 1, and 1.5 mg/100 g body weight), with each treatment conducted in triplicate. The mangrove leaf extract was injected into the experimental animals on the fourth day after stocking. The crabs were fed trash fish at 10–15% of body weight twice daily. Gonadal maturity stage (GMS), gonado-somatic index (GSI), and hepatopancreas-somatic index (HSI) were observed on day 15 post-injection (one individual from each treatment) and day 30 post-injection (all individuals from each treatment). Descriptive analysis indicated that the gonadal maturity stage of the experimental crabs increased across all treatments. The highest GMS and GSI values, along with the lowest HSI value, were obtained in the treatment receiving 1 mg/100 g body weight. Water quality parameters during the culture period remained within the optimal range for mud crab cultivation. Injection of *R. mucronata* leaf extract at a dosage of 1 mg/100 g body weight was found to be the most effective dose for enhancing gonadal maturation and GSI in mud crabs.

Keywords: Bycatch Fish, Crab Apartment, Gonado-Somatic Index, Hepatopancreatic-Somatic Index, Water Quality

ABSTRAK

Kepiting bakau betina merupakan komoditas perikanan bernilai ekonomi tinggi karena potensi gonadnya yang diminati pasar. Hingga saat ini, pasokan pasar masih didominasi oleh hasil tangkapan alam. Kondisi ini mengancam keberlanjutan stok kepiting bakau, baik untuk pasokan pasar maupun kelestarian populasinya di alam. Penelitian bertujuan untuk mengetahui pengaruh ekstrak daun mangrove (*Rhizophora mucronata*) terhadap tingkat kematangan gonad kepiting bakau (*Scylla olivacea*). Penelitian ini menggunakan Rancangan Acak Lengkap dengan mengujikan empat dosis ekstrak daun mangrove (0; 0,5; 1; dan 1,5 mg/100 g bobot tubuh). Setiap perlakuan diberi tiga kali ulangan. Ekstrak daun mangrove diinjeksi pada organisme uji di hari ke-4 pascapenebaran. Organisme uji diberi pakan ikan rucah sebanyak 10-15%/bobot tubuh dengan frekuensi dua kali sehari. Tingkat kematangan gonad (TKG), *gonado-somatic index* (GSI), *hepatopancreas-somatic index* (HSI) diamati pada hari ke-15 (1 individu tiap perlakuan), dan hari ke-30 (semua individu pada setiap perlakuan) pascapenyuntikan. Secara deskriptif menunjukkan TKG kepiting uji pada seluruh perlakuan mengalami peningkatan. TKG dan GSI tertinggi serta nilai HSI terendah diperoleh pada perlakuan dosis 1 mg/100 g bobot tubuh. Parameter kualitas air selama pemeliharaan masih berada pada kisaran yang sesuai untuk pemeliharaan kepiting bakau. Injeksi ekstrak daun *R. mucronata* dengan dosis 1 mg/100 g bobot tubuh merupakan dosis yang tepat dalam meningkatkan kematangan gonad, dan GSI kepiting bakau.

Kata Kunci: Apartemen Kepiting, *Gonado-Somatic Index*, *Hepatopancreas-Somatic Index*, Kualitas Air, Pakan Ikan Rucah

INTRODUCTION

The mud crab is a fishery commodity of high economic value (Fardiyah et al., 2021). This is evident from the market prices per kilogram for the species *Scylla olivacea* in South Sulawesi; for males, prices are IDR 120,000 for the ≥200 to <300 g size range, IDR 150,000 for ≥300 to <500 g, and IDR 170,000 for ≥500 g. In contrast, fully gravid (egg-bearing) females command higher prices: IDR 150,000 for the ≥200 to <250 g range, IDR 240,000 for ≥250 to <300 g, and IDR 330,000 for ≥300 g. According to Syafaat et al. (2021), the market demand for mud crabs increases annually, yet meeting this demand still largely relies on wild-caught specimens.

The extensive commercial exploitation of mud crabs places pressure on natural stocks—a phenomenon exacerbated by the specific demand for females. Market conditions and high selling prices drive massive wild harvesting without regard for the crabs' reproductive potential. Consequently, efforts are needed to accelerate gonad maturation in order to manage resources and support the development of sustainable mud crab aquaculture capable of meeting market demand. One approach to supporting sustainable aquaculture is through hatchery operations, which require an understanding of the gonad maturation process—a process currently hindered by issues regarding maturation stage synchronization and delays (Awaludin et al., 2021; Kasprijo et al., 2017). One approach to addressing this issue is the application of hormones, which are expected to stimulate faster gonad maturation.

Several researchers have reported that plant-derived hormones can enhance gonad maturation in crustaceans, including mud crabs; for instance, Farizah et al. (2017) demonstrated that the use of natural material (karamunting leaves) via injection could accelerate gonad maturation. Subsequent research by Jatiswara et al. (2020) reported that extract from the purwoceng plant could also trigger gonad maturation. Iromo et al. (2023) and Farizah et al. (2017) further noted that bioactive compounds found in the karamunting plant—specifically flavonoids and steroids/triterpenoids—are capable of inducing gonad maturation in mud crabs. Active compounds proven by researchers to trigger gonad maturation in crabs include

flavonoids and steroids (Awaludin et al., 2020; Farizah et al., 2017; Iromo et al., 2023).

The mangrove species *Rhizophora mucronata* is known to contain bioactive compounds—specifically flavonoids and steroids—in its leaves (Egra et al., 2019; Kasitowati et al., 2017; Suciati, 2012), similar to those found in karamunting (Farizah et al., 2017) and purwoceng (Jatiswara et al., 2020). Research regarding the use of active compounds from mangrove leaves to stimulate gonad maturation in mud crabs remains limited, necessitating further study. Therefore, this research aims to examine the effect of mangrove (*R. mucronata*) leaf extract on the gonad maturation stage of female mud crabs (*S. olivacea*).

RESEARCH METHODS

Time and Location

The entire series in this research was carried out for three months starting from October to December 2023. The place of implementation was the Water Quality and Aquatic Biology Laboratory, Faculty of Animal Husbandry and Fisheries, Tadulako University, Palu, Central Sulawesi Province.

Preparation of Test Organisms and Mangrove Leaf Extract (*R. mucronata*)

The mangrove crab (*Scylla olivacea*) used was female (with gonad maturity level 0; TKG-0), measuring 150-250 g, healthy and without defects, brightly colored and complete with body organs (claws, walking legs and swimming legs). Mangrove crabs were obtained from West Bolano Village, Bolano District, Parigi Moutong Regency, Central Sulawesi.

Mangrove leaves were obtained from the coast of Layana Beach, Mamboro Village, Palu City, Central Sulawesi Province. Mangrove leaves (*R. mucronata*) are taken (2nd and 3rd leaves from the tip of the stem), then air-dried under indirect sunlight. Once dry, the mangrove leaves are bladed until smooth. Next, Mangrove (*R. mucronata*) leaf extract was made by maceration using 80% ethanol solvent. Dried mangrove leaves are macerated using 80% ethanol solvent at 5 mL/g of material for 3 times 24 hours at room temperature (18-25°C) (Farizah et al., 2017). After that, filtering is carried out to obtain the filtrate, then evaporated using an evaporator (Iromo et al., 2023), resulting in mangrove leaf extract (*R. mucronata*), which is also known as simplicia.

Container Preparation

The maintenance containers used in the research were a crab box and a four-square fiber tub measuring 120 x 250 x 60 cm^3 (filled with water with a volume of 1,050 L) as water storage containers in the recirculation system. The container is cleaned before use, positioned, filled with water and equipped with a filter system (one top filter unit). After that, the water was circulated for 3 days before stocking the mud crabs.

Distribution and Maintenance of Test Organisms

Mangrove crabs (*S. olivacea*) as the test organism had their weight recorded and placed in each box based on randomization results for all treatments. Mud crabs were reared at a density of 1 fish/box for 34 days (including 4 days before injection). During maintenance, feed is given in the form of trash fish as much as 10-15% per body weight. Frequency of feeding twice a day (06.00 and 18.00). Water quality control (pH, dissolved oxygen/DO, temperature and salinity) is carried out once a week by taking measurements and increasing the volume of water in the recirculation tank if the volume decreases. The level of gonad maturity was observed twice, namely on the 15th day and the 30th day after injection. The treatment doses tested in this study were A (control/without mangrove leaf extract), B (0.5 mg/100 g body weight), C (1 mg/100 g body weight), and D (1.5 mg/100 g body weight).

Determination of the highest dose in this study refers to the results of research by Heryanto (2022), namely 1.5 mg/100 g body weight of the test organism.

Injection of Mangrove Leaf Extract into Test Organisms

The research was designed experimentally using a completely randomized design (CRD) to test various doses of mangrove leaf extract. The dosage treatments are; 0 mg/without mangrove leaf extract/control (A); 0.5 mg (B); 1 mg (C); and 1.5 mg (D). Before injection, mangrove leaf extract (simplisia) is dissolved in 100 µL NaCl solution for each treatment dose by vortexing for approximately 2 minutes. Injection at the base of the swimming legs using each syringe (1 mL in size) at an angle of 45° for each test organism (Farizah et al., 2017). Injection once (4th day after stocking). The same process for the control (0 mg/without mangrove leaf extract) is only injected with 100 µL of NaCl solution. Each treatment was given 4 repetitions.

Observed Variables

Gonad Maturity Level (TKG)

The gonad maturity level (TKG) of the test organisms is seen macroscopically (Farizah et al., 2017; Iromo et al., 2023). Gonads were taken from one test organism per treatment on day 15 (observations focused on color and gonad volume). The next observation was carried out at the end of maintenance (day 30 post-injection) for all test organisms in all treatment groups using the same procedure as the first observation (day 15).

Gonadosomatic and Hepatopancreas-somatic Indices

Measurements of the gonadosomatic index (GSI) and hepatopancreas-somatic index (HSI) were conducted at the end of the rearing period (day 30 post-injection). Measurements involved weighing each test crab across all replicates for every treatment. Subsequently, the gonads and hepatopancreas were removed from the test crabs and placed in separate containers. The weights of the mangrove crab gonads and hepatopancreas were recorded for all replicates in each treatment. GSI values were calculated based on the study by Iromo et al. (2023), while HSI values were calculated based on the study by Farizah et al. (2017).

$$\text{GSI (\%)} = \frac{\text{Gonad weight (g)}}{\text{Test organism weight (g)}} \text{ x } 100$$

$$\text{HSI (\%)} = \frac{\text{Hepatopancreas weight (g)}}{\text{Test organism weight (g)}} \text{x } 100$$

Absolute Weight Growth

Absolute weight growth was determined by weighing the test crabs in each replicate for all treatments on days 10, 20, and 30 of the post-injection rearing periods. The absolute weight gain of the mangrove crabs was calculated using the following equation: mean weight of test organisms at the end of rearing (g) – mean weight of test organisms at the start of rearing (g) (Rahman & Safir, 2018).

Data Analysis

The data obtained—comprising GSI, HSI, gonad maturity stage, absolute weight growth, and water quality—were analyzed descriptively.

RESULT

Mud Crab Gonad Maturity Stage

At the start of the rearing period (prior to injection), the gonad maturity stage (GMS) of

the mud crabs was GMS 0 (Figure 1a). After 15 days of injection with mangrove leaf extract, the mud crabs (the test organisms) exhibited signs of gonad maturation. Descriptively, mud crabs in Treatment A (control/0 mg per body weight) and Treatment B (dosage of 0.5 mg per body weight) showed GMS II—though Treatment B displayed a slightly larger gonad volume (Figures 1b & c). Crabs in Treatment C (dosage of 1 mg per body weight) had reached GMS III (Figure 1d), while those in Treatment D (dosage of 1.5 mg per kg body weight) were in the transitional phase between GMS II and GMS III (Figure 1e).

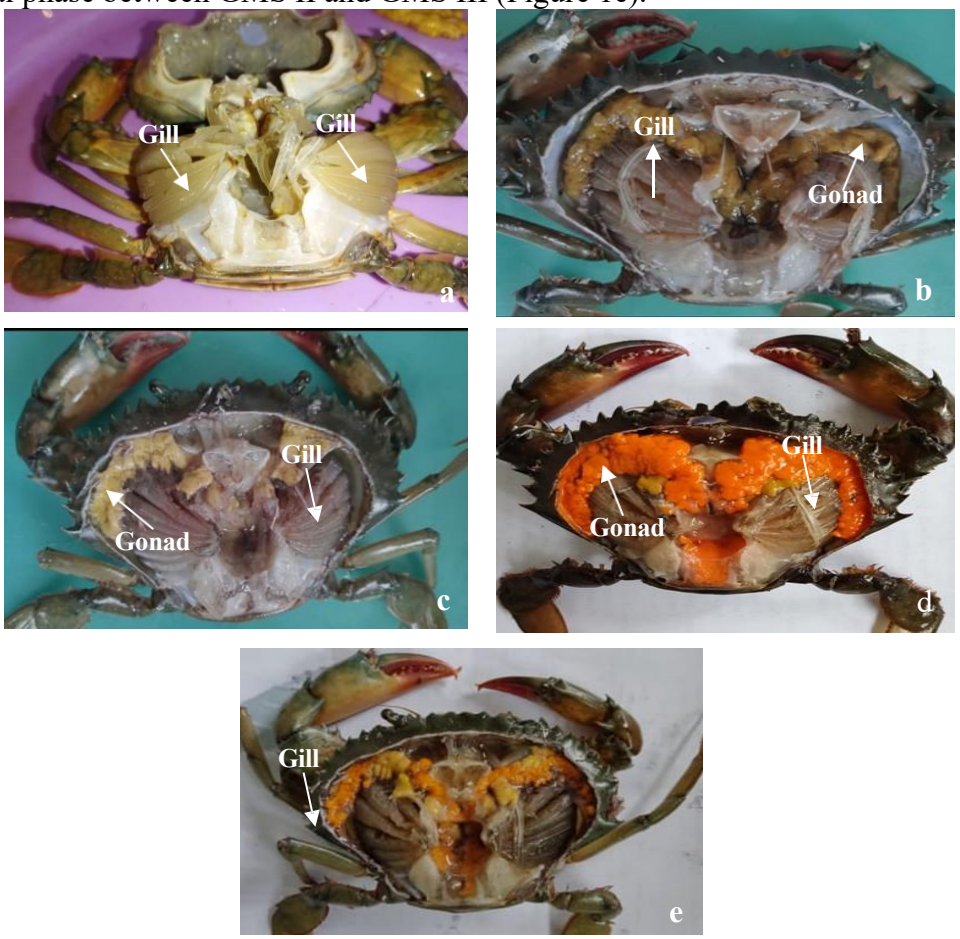

Figure 1. Gonad Morphology of Mud Crabs 15 Days After Injection with Mangrove Leaf Extract; a) Gonad Appearance Prior to Injection; b) Control/0% Dosage; c) 0.5 mg Dosage; d) 1 mg Dosage; and e) 1.5 mg Dosage (All Dosages per 100 g of Mud Crab Body Weight)

After 30 days of rearing following the injection of mangrove leaf extract, the gonad maturity stages of the mud crabs (*S. olivacea*) indicated varying degrees of development across the different treatments. Descriptively, mud crabs in treatment A (control/0 mg per body weight) had reached Gonadal Maturity Stage (GMS) II with optimal gonad volume (Figure 1a); those in treatment B (dose of 0.5 mg per body weight) were at GMS II transitioning to GMS III (Figure 1b); and those in treatments C (dose of 1 mg per body weight) and D (dose of 1.5 mg per body weight) had reached GMS III, with treatment C showing more advanced progress in gonad volume (Figures 1c–d).

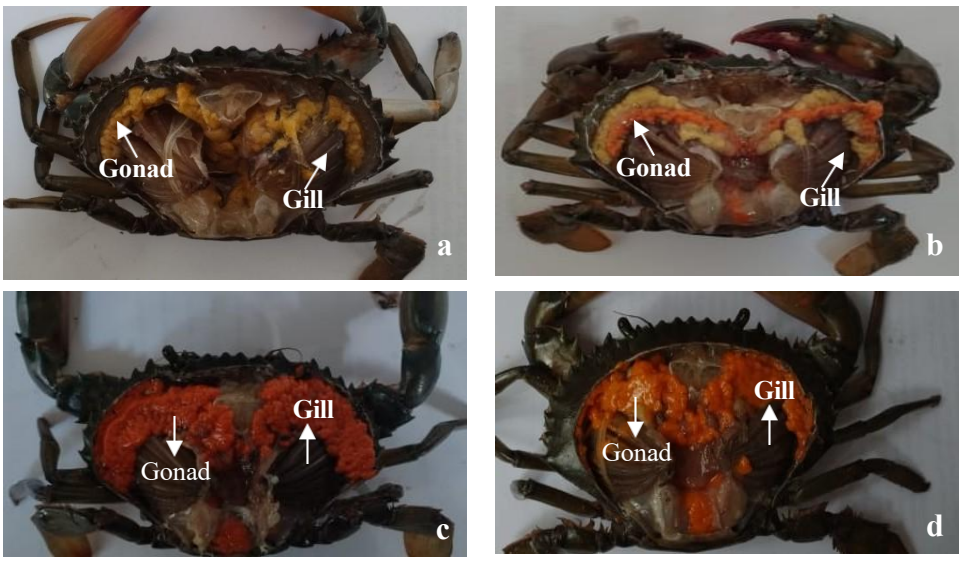

Figure 2. Gonad Morphology of Mud Crabs on Day 30 Post-Injection with Mangrove Leaf Extract; a) Control/0% Dose; b) 0.5 mg Dose; c) 1 mg Dose; and d) 1.5 mg Dose (All per 100 g of Mud Crab Body Weight)

Gonado-somatic Index (GSI)

The Gonado-somatic index (GSI) of mud crabs on days 15 and 30 post-injection with mangrove leaf extract showed an increase following a similar pattern across all treatments. The GSI values on days 15 and 30 post-treatment, ranked from highest to lowest, were observed in the following treatments: 1 mg dose (10.42%), 0.5 mg dose (6.84%), 1.5 mg dose (6.53%), and control/0% dose (4.07%) (all per 100 g of mud crab body weight). In general, treatments receiving mangrove leaf extract (*R. mucronata*) yielded higher values compared to the control treatment (Table 1).

Hepatopancreas-somatic Index (HSI)

The hepatopancreas-somatic index (HSI) values for mud crabs on days 15 and 30 post-injection with mangrove leaf extract are presented in Table 1. On day 15 post-treatment, the HSI values, ranked from highest to lowest, were observed in the following treatment groups: 1 mg (1.03%), 0.5 mg (0.45%), 1.5 mg (0.32%), and the control/0 mg (0.13%)—values expressed per 100 units of test organism body weight. Subsequently, on day 30 post-injection, the HSI values of the mud crabs decreased, with the lowest values generally observed in the crabs treated with mangrove leaf extract (Table 1).

Table 1. Gonado-somatic Index (GSI) and Hepatopancreas-somatic Index (HSI) of Mud Crabs Treated with Mangrove Leaf Extract on Days 15 and 30 Post-Injection

Observed Variables	Dosage Treatment of Mangrove Leaf Extract per 100 g of Test Organism Body Weight			
	Control/0 mg	0.5 mg	1 mg	1.5 mg
GSI – 15 days (%)	4.07	6.84	13.17	6.53
HSI – 15 days (%)	0.13	0.45	1.03	0.32
GSI – 30 days (%)	5.15±1.04	9.57±3.87	10.42±5.41	9.34±4.57
HSI – 30 days (%)	0.15±0.08	0.13±0.04	0.11±0.06	0.12±0.03

*Different superscript letters in the same row indicate significant differences between treatments (P<0.05).

Water Quality

The mud crab rearing process utilized a 24-hour recirculation system; pumps circulated water from the reservoir tank to each rearing box, while water flowed via diffusion into the filtration and reservoir tanks. Consequently, water quality parameters for each treatment were maintained at levels suitable for mud crab rearing. The values of water quality parameters in the rearing medium are presented in Table 2.

Table 2. Water Quality Parameters During the Study

No.	Parameter	Measurement Results	Reference Value	Reference
1.	Temperature (°C)	27 – 29	25-35	(Sari et al., 2022)
2.	pH	6.54 – 7.24	7.7-7.9	(Sari et al., 2022)
3.	Salinity (ppt)	25 – 29	15-30	(Fujaya, 2011)
4.	Dissolved oxygen (ppm)	5.5 – 7.1	5.7-6.0	(Suprapto et al., 2014)

DISCUSSION

Descriptively, the application of mangrove leaf extract influenced gonad maturation in mud crabs (*Scylla olivacea*). This was evidenced by faster gonad maturation across all mangrove leaf extract treatments compared to the control. Prior to treatment, the crabs were at Gonad Maturity Stage (TKG) 0; however, 15 days post-treatment, they showed progression to higher stages, indicated by changes in gonad color and volume (Figure 1 and Table 1). On day 15 post-injection, crabs in Treatment A (control/0 mg) exhibited light yellow gonads with low volume (GSI: 4.07%), indicating a transition toward TKG II. Crabs in Treatment B (0.5 mg dose) showed a similar gonad color to the control but a higher gonad volume (GSI: 6.84%); they also reached the same maturity stage as the control group by day 15.

Gonad maturity levels in the mud crabs increased further 30 days after injection across all treatments. Based on gonad color (Figure 2) and volume (indicated by the gonadosomatic index), the ranking of maturity levels—from highest to lowest—was observed at doses of 1 mg (C), 1.5 mg (D), 0.5 mg (B), and 0 mg (A/control) per 100 g of body weight. The higher maturity levels observed across all mangrove leaf extract treatments are likely attributable to the presence of active compounds, specifically triterpenoids and steroids. These compounds are known to enhance reproductive performance in mud crabs; this aligns with findings by Farizah et al. (2017) regarding karamunting leaf extract, which contains bioactive triterpenoids and steroids proven to accelerate ovarian maturation in mud crabs (*S. olivacea*).

Steroids are derivatives of triterpenoids and are classified into progestins and estrogens (Farizah et al., 2017). Both types of compounds play a role in gonad maturation in crustaceans, including the mangrove crab. Gunamalai et al. (2006) reported that the use of progestins (17α-hydroxyprogesterone, 20α-hydroxyprogesterone, and 6β-hydroxyprogesterone) and estrogens (17β-estradiol and estrone) has been shown to enhance gonad development in crustaceans. The mechanism of gonad maturation begins with the entry of steroid hormones (progestins and estrogens) into the bloodstream and their transport to the hepatopancreas; there, they bind to receptors to stimulate protein synthesis, specifically for the production of vitellogenin.

High-intensity vitellogenesis directly increases the amount of vitellogenin produced, thereby accelerating gonad maturation in the crabs. The reproductive physiological processes demonstrated in this study indicate that gonad maturation is achieved more rapidly as the treatment dosage increases (up to 1 mg), a trend supported by the rise in GSI values (Table 1). The increase in GSI—an indicator of gonad maturation—is directly associated with a decrease in HSI values (Farizah et al., 2017).

The observed decrease in hepatopancreas values reflects nutrient mobilization toward the gonads, driven by the active compounds (triterpenoids and steroids) in the mangrove leaf

extract that stimulate gonad maturation in the test crabs. As noted by several researchers, the hepatopancreas serves as a metabolic hub and a site for the absorption and storage of nutrient reserves—such as lipids and glycogen—which are subsequently utilized to support metabolic processes, particularly during molting and reproduction, including gonad development and maturation (Abubakar et al., 2025; Sari et al., 2022; Wang et al., 2014).

Regarding the gonad maturation observed in the 1.5 dosage treatment, the lower maturation level suggests that the dosage used was excessive, resulting in a diminished response. This aligns with findings reported by Farizah et al. (2017), indicating that higher doses of the extract can inhibit gonad maturation in mud crabs. Meanwhile, the control group exhibited a similar pattern of gonad maturation throughout the experiment but at a slower rate, due to the absence of stimulants for the maturation process. Furthermore, water quality measurements taken during the study indicated that all treatments remained within the optimal range for mud crab rearing. A closed recirculating system was employed for water quality management, with strict monitoring to ensure parameter stability in accordance with reference standards (Table 2).

CONCLUSION

Injection of *Rhizophora mucronata* mangrove leaf extract into mud crabs was shown—based on descriptive analysis—to enhance gonad maturity and the gonadosomatic index, while reducing the hepatosomatic index. The dosage effective for accelerating gonad maturity in mud crabs is 1–1.5 mg per 100 g of the test organism's body weight.

ACKNOWLEDGEMENT

We extend our gratitude to the National Research and Innovation Agency (BRIN) and the Indonesia Endowment Fund for Education Agency (LPDP) for providing research funding under contract numbers 127/IV/KS/11/2022 and 2915/UN28.2/PL/2022.

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