The effect of a recirculation aquaculture system on the reproductive performance of female mud crabs (*Scylla serrata*)

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Abstract. One of the problems in a mud crab hatchery is the low reproductive performance of the broodstock, even when the selection process has been carried out properly. Improving water quality with an aquaculture recirculation system (RAS) is expected to improve the reproductive performance of broodstock and to obtain descendants with high quality. The purpose of this study was to determine the effect of RAS on the reproductive performance of female mud crabs (*Scylla serrata*). The study was conducted by comparing two treatments, namely RAS and conventional systems (CS). The results of data analysis using Student's t-test showed significant differences ($p<0.05$) between the two treatments in the assessment of gonad maturity level, percentage of maturity, latency period, percentage of spawning, and survival rate. In the assessment of the incubation time parameters of the two treatments, the results were not significantly different ($p>0.05$). The RAS system with a biofilter can maintain and improve the water quality of the broodstock culture environment, so that it can optimize and improve the reproductive performance of female mud crabs *S. serrata*.
Key Words: broodstock, conventional systems, RAS, reproductive performance.

Introduction. Introduction. Mud crabs of the genus Scylla are a fishery commodity with economic value in Indonesia, in great demand by both domestic and international markets (Iromo et al 2018; Siahainenia 2008; Yamin & Sulaiman 2011). This commodity can be commercialized live, frozen or canned. Based on data from the Statistics Indonesia, exports of crabs and shellfish in 2015 reached 109614.507 tons with an export value of 266825892 USD (Export Statistics Subdirectorate 2015). Furthermore, Hungria (2017) reported that the total world crab production in 2015 reached 1300000 tons. Until now, Indonesia has only been able to meet about 25% of the market demand for mud crabs (Aisyah et al 2019). The high price and demand increase the exploitation of mud crabs and there is concern that overexploitation can occur (Heasman & Fielder 1983; Yi et al 2009). Up to now, the increase in production has not been carried out intensively because most of it is the result of capture fisheries, being limited by the availability of stocks in nature (Herliany & Zamdial 2015). Increasing production intensively and sustainably can be done by cultivating in ponds. Cultivation development is still constrained by the availability of fingerlings, most of which are fishermen's catch. Efforts to supply fingerlings through hatcheries have been carried out, but they have not yet made a significant contribution (Djunaidah et al 2003).

One of the obstacles in mud crab hatchery is represented by the limitations of female maturity (Millamena & Quinitio 2000). The broodstock used in hatchery units is formed by adult females, collected when they attained mature ovaries in nature (Yi et al 2009; Waiho et al 2018). Therefore, an effective cultivation method is needed to improve the maturation of female ovaries and spawning time in captivity, in order to support sustainable fingerlings production. Several previous studies reported that the reproductive performance of female broodstock in crustaceans is influenced by factors including genetics, individual physical status, nutrition and quality of the culture water (Perez-Velazquez et al 2001). Genetic-related research has been carried out by Shi et al (2018) and it was concluded that the sex-specific SNP markers identified in the mud crab species *S. paramamosain, S. tranquebarica*, and *S. serrata* showed female heterogamety and male homogamety, thus providing evidence for the WZ/ZZ sex-determination system. The results of other studies related to nutrition to increase the reproduction of mud crab broodstock showed that artificial feed was comparable to fresh feed, where to meet the needs of essential lipids, fresh feed is combined with 10% artificial feed (Djunaidah et al 2003; Alava et al (2007).

Poor water environment in the culture media can cause low reproductive performance, such as disturbing of the ripening time and spawning. Fluctuating environmental conditions can lead to egg abortion and the death of the mother. Environmental manipulation is needed to maintain cultivation conditions optimal for improving the reproductive performances of female mud crabs. The aquaculture recirculation system (RAS) is a technology that can create stable water parameters and quality, and optimal conditions during culture (Hastuti et al 2019; Ren et al 2020). The results of the literature review show that RAS technology in the development of a mud crab culture has been widely used, but not necessarily in relation to reproductive performance. Thus, it is necessary to research the effect of RAS on the reproductive performance of female mud crabs.

The results of this research are expected to be useful in the development of science, especially in the field of fingerlings engineering and reproduction of mud crabs, and can provide information for farmers, especially in environmental management for the maintenance of mud crab broodstock in hatcheries. Therefore, the main objective of this study was to determine the effect of RAS on the reproductive performance of female mud crab *S. serrata*.

Material and Method

Description of the research location. This research was conducted from September to October 2020 at the Center for Brackishwater Aquaculture Development (CBAD), Jepara, Indonesia (Figure 1). CBAD Jepara is the technical implementation unit of the Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries of the Republic of Indonesia. CBAD conducts research and engineering activities on brackishwater fish species including crustaceans (shrimp, mud crabs and blue swimming crabs), milkfish, brackish water tilapia, seaweed, and laboratory services (fish health laboratory, environmental chemical physics laboratory and natural food laboratory).

Animals. Adult female mud crabs were obtained from fishermen or collectors in Jepara Regency, Central Java Province, Indonesia. Identification of mangrove crab species was carried out according to the morphological description by Keenan et al (1998). The characteristics of 20 selected female mud crabs were defined as complete limbs, no defects, no biofouling, active response when the swimming legs are held, carapace width of at least 110 mm, initial body weight of at least 300 g, and ovarian maturity level of stage 3. Observation of maturity level of ovaries was performed visually, on the gonads, by pushing the first abdominal segment bordering the carapace (Djunaidah et al 2003; Pattiasina et al 2012). Orange young female mud crabs are considered to be breeding and have reached stage 3 (Wu et al 2020). Transportation was carried out using a dry system on the same day to CBAD, Jepara. Before acclimatization, mud crab broodstock was disinfected with a 25 mg L^{-1} concentration of formalin solution for 30 min, then acclimatized for 3 days, without any feed. Furthermore, the crabs were transferred to the research tank.

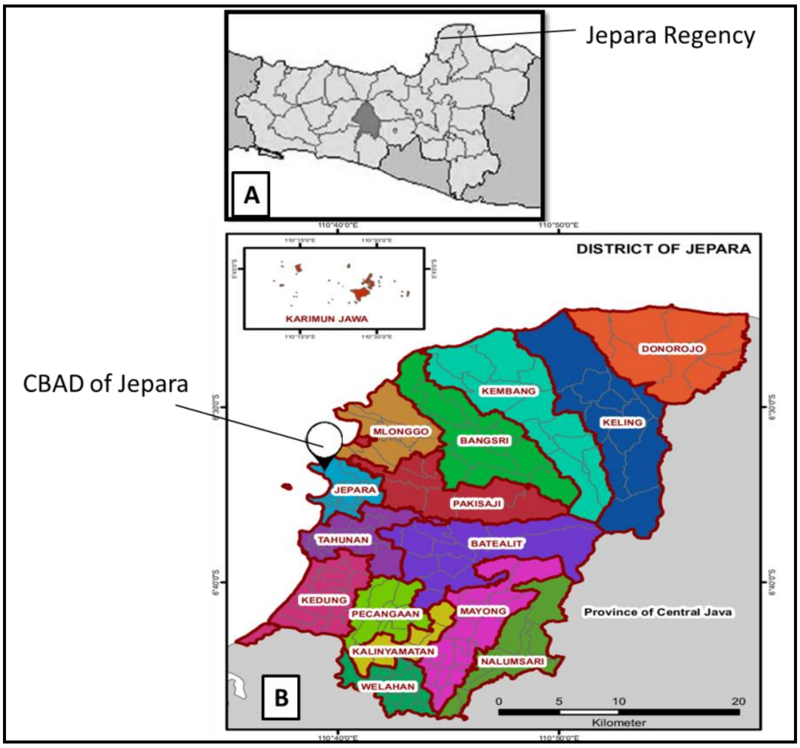

Figure 1. Research location in CBAD Jepara; a - location of Jepara Regency in Central Java, Indonesia; b - location of CBAD in Jepara Regency.

Experimental design. Experiments were carried out by comparing two different systems, namely a recirculation aquaculture system (RAS) with a biofilter (Figure 1) and a conventional system (CS) (Figure 2). RAS consisted of 10 broodstock tanks equipped with fine sand substrate and aeration system. The broodstock tank was connected by a water outlet pipe to the first biofilter tank containing 500 L bio balls. Furthermore, the first biofilter tank is connected to the second biofilter tank, designed to consist of 300 L of ginger coral, 6 water lifts, and a pump connected to the inlet pipe. The inlet water pipe was equipped with a control valve to regulate the water pressure and drain it to the control tank, which was placed above the second biofilter tank. The control tank functioned to adjust the volume of water in the second biofilter bath, to avoid overflowing. Water from the control tank flows through a small hole in the bottom of the tank.

The operation of RAS was initiated by culturing the nitrifying bacteria in a biofilter tank, which was carried out at least 7 days before the experiment. Operations began with running the entire RAS. Nitrifying bacteria *Nitrobacter* and *Nitrosomonas* were obtained from the Fish Health Laboratory of CBAD Jepara. Measurement of water quality parameters consisting of alkalinity and ammonia was carried out before bacterial culture. The optimal standard for water quality is an alkalinity of 200 mg L^{-1} to 250 mg L^{-1} and an ammonia level >3 mg L^{-1}. Lower alkalinity values can be increased by adding sodium bicarbonate ($NaHCO_3$) 150 mg L^{-1}, and ammonia levels can be increased by adding 5 mg L^{-1} ammonium chloride (NH_4Cl). Nitrifying bacteria consisting of *Nitrobacter* and *Nitrosomonas* with a bacterial density of 104 cfu mL^{-1}, and a volume of 50 mL were inoculated into the biofilter tank. Observation of water quality parameters consisting of alkalinity and ammonia was carried out daily to ensure that nitrifying bacteria were working properly in the system. A decrease in the value of ammonia concentration to <0.1 mg L^{-1} is an indicator that the bacteria are functioning and working well in the nitrification process. Furthermore, the adjusted female mud crabs could be placed in the mother trough to observe their reproductive performance.

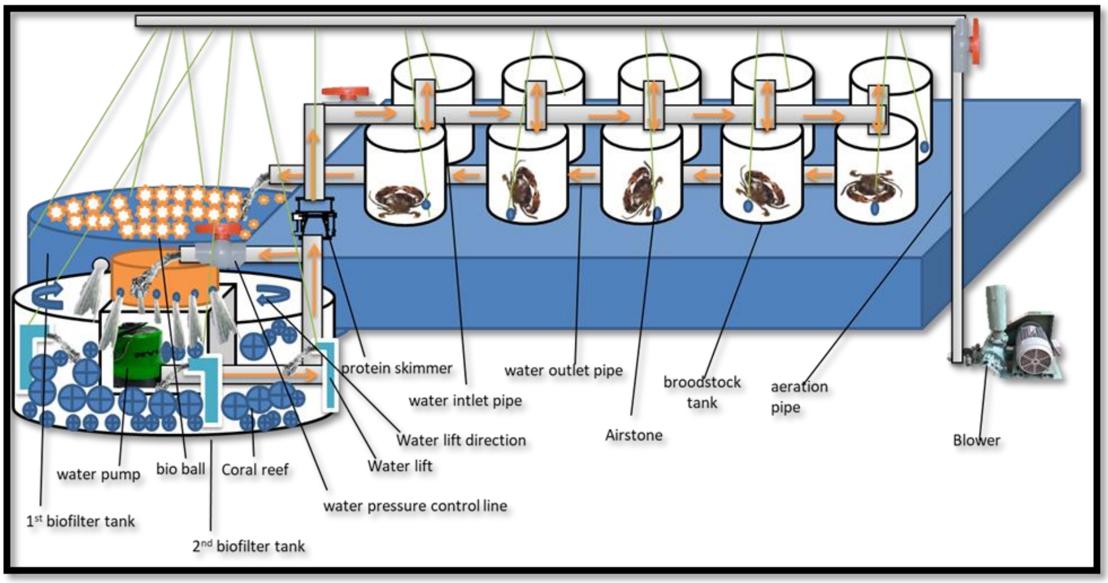

Figure 1. Schematic design of a recirculating aquaculture system (RAS).

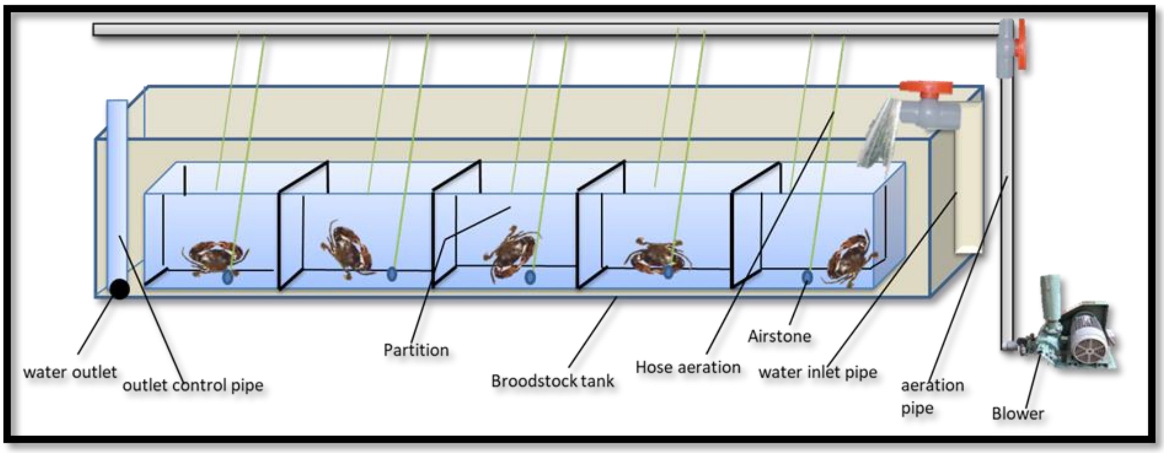

Figure 2. Schematic design of a conventional system (CS).

The CS experiment was carried out on a square concrete tank measuring 5×2×1.1 m equipped with a water inlet pipe, water outlet, and aeration equipment. Furthermore, 10 boxes of wooden slats were installed in the concrete tank with a size of 0.6×0.6×0.8 m. The bottom of the tank was covered with a fine sand substrate with a thickness of 0.05 m. The water level in the broodstock tank during the experiment was 0.4 m. Water was changed 100% every 3 days before feeding.

The water salinity in all treatments was 30 ppt. The feed given was blood clams (*Anadara granosa*) with a feeding dose of 10% of biological material body weight. Feeding was done in the morning and evening with a ratio of 30:70%. The remaining feed was collected and discarded before the next feeding.

Reproduction parameters. The gonad maturity level was observed every 3 days during the experiment. The maturity level of the gonads was recorded during the experiment and accumulated until the end of the experiment. The observation method was based on the report of Djunaidah et al (2003) and Pattiasina et al (2012). Gonad maturity level assessment was done by observing color development based on previous research by Wu et al (2020): stage 1 - translucent to creamy white; stage 2 - creamy; stage 3 - light yellow; stage 4 - light yellow to bright orange; and stage 5 - light orange to reddish.

Maturity percentage is the ratio between the number of mature females in stage V and the number of females in the treatment (Pamungkas et al 2014). The latency period is the duration of time the female is mature until spawning (Djunaidah et al 2003). The percentage of spawning is the percentage between the number of females spawning and the number of females in the treatment (Pamungkas et al 2014; Maulana et al 2017). The incubation time is the duration of incubation required for the embryo to hatch (Herlinah & Septiningsih 2015). The survival rate is the percentage of broodstock that lived to the end of the experiment (Maulana et al 2017).

Analysis of water quality parameters. The daily water quality parameters observed were dissolved oxygen (DO), pH, temperature, and alkalinity. Observations of total ammonium nitrogen (TAN) were carried out once every three days along with other parameter measurements. Measurement of temperature and dissolved oxygen was conducted using a DO meter (YSI 58, Yellow Springs Instruments co. Inc., USA), pH using a portable pH meter (Meterlab PHM 201, Radiometer Analytical, SA, France), salinity using a hand refractometer (Atago S/mill - E - Japan), TAN and alkalinity using the titration method in the Environmental and Residual Chemistry Physics Laboratory, CBAD, Jepara.

Statistical analysis. Data are presented as means ±SD. The parameters of survival rate, latency period, and incubation time were analyzed by Student's t-test. Before analysis with Student's t-test, the Shapiro-Wilk test was performed to test the normality and homogeneity of data variance, respectively, and $p<0.05$ was considered significant. All statistics were performed by IBM SPSS software, version 26. Data on the percentage of maturity, percentage of spawning, and water quality were analyzed using experimental methods. The data were processed using Microsoft Excel 2010 and discussed descriptively.

Results and Discussion

Gonad maturity level. Observations during the experiment showed the effect of RAS treatment on the maturity level of female mud crab gonads. Observation data are presented in Tables 1 and 2. It can be seen that the crabs from the RAS treatment reached gonad maturity stage 4 faster, namely on day 6 with the percentage of gonad maturity reaching 71.03±4.18%. In the CS treatment, maturity stage 4 was obtained on day 12 with a percentage of gonad maturity of 66.67±57.74%. Furthermore, maturity stage 4 of 100% was reached on day 9 in the RAS treatment. At maturity stage 5, RAS treatment also showed better results, where on day 12, the percentage of gonad maturity had reached 68.54±4.58% (Table 3). Crabs in the CS treatment reached ovarian maturity stage 5 on day 18, amounting to 14.28%. On day 21, female mud crabs in the RAS had all spawned, while in the CS, this was achieved on day 30.

Table 1

Cumulative percentage of maturity stages of mud crab females (stage 3)

Treatment	*Cumulative percentage of maturity stages of mud crab females (stage 3)*			
	day-0	*day-3*	*day-6*	*day-9*
CS	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
RAS	100.00±0.00	100.00±0.00	28.97±4.18	0.00±0.00

Table 2

Cumulative percentage of maturity stages of mud crab females (stage 4)

Treatment	*Cumulative percentage of maturity stages of mud crab females (stage 4)*					
	day-6	*day-9*	*day-12*	*day-15*	*day-18*	*day-21*
CS	0.00±0,00	0.00±0.00	66.67±57.74	76.19±41.24	86.31±1.03	6.67±11.56
RAS	71.03±4.18	100±0.00	28.97±4.18	0.00±0.00	0.00±0.00	0.00±0.00

Maturity percentage. The percentage of mature female mud crabs during the experiment is presented in Figure 1. The maturity of female mud crabs in stage 5 in RAS treatment was achieved on day 12, with a percentage of 68.54±4.58% and reached a percentage of 100% on day 15. Crabs in the CS treatment reached maturity stage 5 on day 18, at 14.28±0.00%, and all females reached maturity stage 5 on day 24.

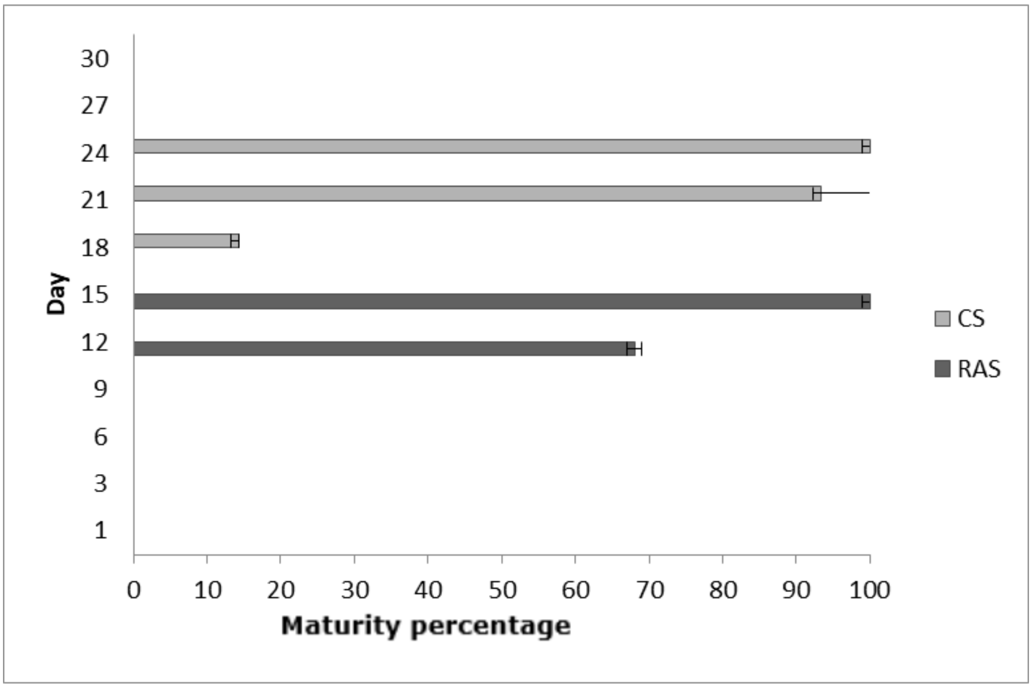

Figure 3. Comparison of the percentage of mature female mud crabs in RAS treatment and in a conventional system during the experiment; bars represents standard error.

Latency period. The reproductive performance was related to the latency period as shown in Table 4. The results of data analysis showed that the latency period of RAS and CS treatments was significantly different ($p<0.005$). The latency period was shorter in RAS than in CS. In RAS, the latency period was 16.84±0.32 days, while in CS, it was 26.3±0.09 days.

Table 4

Mean latency period, incubation time and survival rate of mud crab females during the experiment

	CS	*RAS*	*Statistics*
Latency period (day)	26.30±0.09	16.84±0.32	p<0.05
Incubation time (day)	12.11±0,69	11.67±1.00	p>0.05
Survival rate (%)	43.33±5.77	83.33±5.77	p<0.05

Percentage of spawning. The percentage of spawning is presented in Figure 4. The results showed that the RAS affected spawning time, where on day 15 the percentage of spawning had reached 68.98±6.45% and reached 100% on day 24. Spawning in CS was slower, reaching 6.67% on day 21 and 100% on day 33.

Incubation time. The results are presented in Table 4. The data obtained showed no significant difference ($p>0.05$) for incubation time. RAS incubation time was 11.67±10 days, while CS incubation time was 12.11±0.69 days.

Survival rate. The survival rate of female mud crabs in the two treatments showed a significant difference ($p<0.05$) as presented in Table 4. RAS obtained a higher survival rate, reaching 83.33±5.77%, whereas the survival rate obtained in CS was 43.33±5.77%.

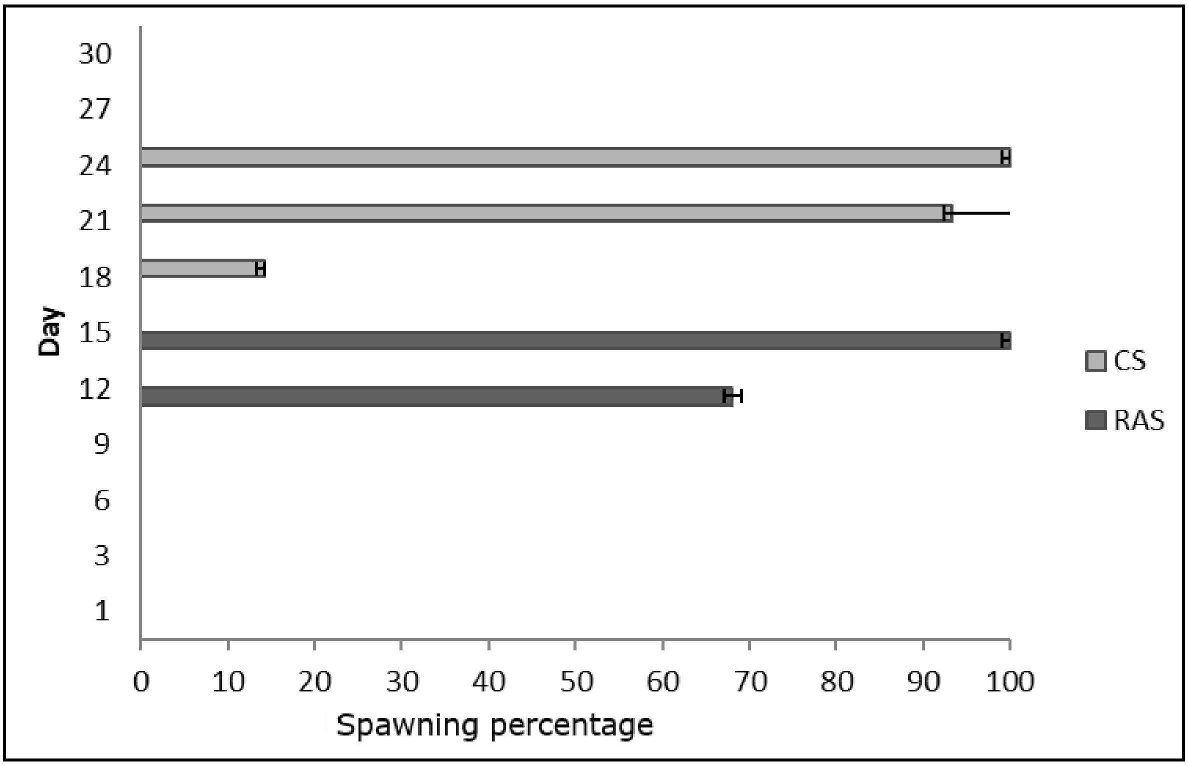

Figure 4. Comparison of the percentage of spawning female mud crabs in the two growth systems; the bars represent the standard error.

Water quality parameters. The water quality parameters during maintenance are presented in Table 5.

Table 5

Average results of water quality measurements during the experiment

Variable	*Treatment*		*Statistics*
	CS	*RAS*	
Temperature (°C)	28.52±0.07	29.41±0.87	p>0.05
pH	8.53±0,02	7.95±0.14	p<0.05
Alkalinity (mg L^{-1})	91.12±10.19	191.52±11.11	p<0.05
TAN (mg L^{-1})	3.14±0.10	0.12 ±0.10	p<0.05
DO (mg L^{-1})	5.32±0.56	5.27 ±0.13	p>0.05

The results showed that RAS had a significant effect on the reproductive performance of female mud crabs. The results of the evaluation of female mud crab reproduction for the gonad maturity stage showed a significant difference, where the RAS on day 6 had reached maturity stage 4, with the percentage of maturity reaching 71.03±4.18%, and 100% on day 9. In CS, maturity stage 4 was reached on day 12, with the percentage of maturity reaching 66.67±57.74%. Ripening (stage 5) in RAS requires a shorter time than in CS. Crabs in RAS reached maturity stage 5 on day 12 with the percentage of maturity reaching 68.54±4.58%, and 100% on day 15. Stage 5 in CS was only reached on day 18, with a percentage of maturity of 14.28%, and reached 100% on day 24. In previous studies with hormone injection treatment and different feeds reported by Pattiasina et al (2010) and Herlinah & Septiningsih (2015), gonad maturation took 18-21 days.

The latent period of the two treatments showed a significant difference ($p<0.05$), where the latency period in RAS was shorter. Research by Djunaidah et al (2003) reported that the latency period of mud crab broodstock with feed treatment was 21-26 days. The spawning percentage parameter also showed a significant difference ($p<0.05$). Female mud crabs in RAS spawned faster, on day 15, with a spawning percentage of 68.98±6.45% and overall spawning on day 24. CS treatment required a slower spawning time.

The survival rate during the experiment was higher in RAS than in CS ($p<0.05$). The reproductive performance parameters for incubation time showed insignificant differences ($p> 0.05$). The incubation time obtained was slower than in previous studies, where the incubation time ranged from 8 days to 10 days (Djunaidah et al 2003).

The results of testing the reproductive performance parameters during the experiment showed significant differences between treatments, indicating that water quality has an important role in the maturation and spawning process of female mud crabs. In RAS, the water quality conditions remained stable and there were no drastic fluctuations or changes during maintenance. These results are consistent with the report of Hastuti et al (2016), who stated that the use of a recirculation system during maintenance aims to maintain the stability of water quality, and reduce media water change by flowing continuously through several filtration systems. Mud crabs require more stable water quality conditions for gonadal maturation and spawning. In the wild, female mud crabs will migrate after mating from the fluctuating intertidal zone to the sea, which is more stable to changes in water quality (Waiho et al 2015; Alberts-Hubatsch et al 2016). This is different in CS, where water changes were carried out every 3 days, resulting in the crabs having to adapt to fluctuations in water quality. Environmental changes will affect reproductive performance, because the amount of energy needed for the adaptation process is greater than the energy for metabolic processes, and it can even cause the death of mud crabs (Hastuti et al 2016).

The pH value during the RAS experiment was 7.95±0.14. The pH value is the main parameter in RAS because it affects the biofilter nitrification rate. The optimal pH value range for nitrification ranges from 7 to 9, where *Nitrosomonas* will function optimally at pH 7 to 9 and *Nitrobacter* at pH 7.2 to 8.2 (Chen et al 2006). The range of pH values suitable for mangrove crab life is in the range of 7 to 9 (Shelley & Lovatelli 2011; Alberts-Hubatsch et al 2016; Karim et al 2015). The pH value in the CS was in the range of 8.53±0.02. Hastuti et al (2016) stated that pH 8 affected glucose values, being higher than in pH 7. The high glucose values indicated that crabs were experiencing high stress. This is the cause of egg abortion during spawning, egg release during the incubation period, and relatively long spawning time. Apart from pH, another water quality parameter that affects RAS performance is alkalinity. Alkalinity is a measure of the buffer capacity of an aquatic system. Nitrification is an acid generation process, and if the biofilter system water is poorly buffered, the system's pH will be low and affect the biofilter performance. The water alkalinity value in RAS during maintenance was 191.52±11.11 mg L^{-1}, higher than that in the CS, with a value of 91.12±10.19 mg L^{-1}. This value is still above the minimum alkalinity limit for nitrification, which is 75 mg L^{-1} (Gujer & Boller 1986).

The results showed that the TAN concentration in the RAS treatment could be controlled, and maintained at 0.12±0.10 mg L^{-1}. In CS, the TAN value was 3.14±0.10 mg L^{-1}. The TAN concentration is a key parameter of water quality in RAS operations and a limiting factor for the rate of biological and substrate nitrification. The minimum TAN concentration in RAS is 0.05 mg L^{-1} (Chen et al 2006). TAN concentration is an important factor in the aquaculture environment, and can become a serious problem if there is a sudden increase due to changes in water quality factors, such as temperature, pH, salinity, ionic charge, and DO (Royan et al 2019). An increase in TAN above the threshold can cause poisoning because it will interfere with the binding of oxygen in the blood, blood pH, and produce enematic reactions, affect membrane stability or even cause death of aquatic animals. In addition, it can also inhibit growth and reproduction (Sutomo

1989). Control of TAN values in waters can be done by using microorganisms through the nitrification process. The DO concentration value still supports the nitrification process in the RAS treatment. The minimum DO concentration needed to achieve nitrification is 2 mg L^{-1} and optimal concentrations are above 4 mg L^{-1} (Ranjan et al 2019). DO values during the study in RAS had an average of 5.27±0.13 mg L^{-1}, lower than in the CS, with an average of 5.32±0.56 mg L^{-1}. DO values are lower in the RAS treatment because some of the DO has been used for the nitrification process.

Conclusions. In this study, it appears that poor water quality affects the reproductive performance of mud crab females, and conversely, optimal and good water quality management within the RAS can provide a stable high-quality water environment to improve the reproductive performance of female mud crabs. Optimal reproductive performance in cultivation is very important to produce quality fingerlings consistently and sustainably. The recirculation aquaculture system with biofilter has a significant effect on improving the reproductive performance of female mud crabs.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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