Redclaw, *Cherax quadricarinatus* sex-separated rearing strategy enhances reproduction in females

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ABSTRACT

Redclaw crayfish *Cherax quadricarinatus*, a tropical freshwater species, native to Northern Australia and is regarded as a promising species for global expansion of aquaculture. However, poor female fertility is a hindrance towards the commercial production of redclaw. The present study investigated if dissociated; pre-exposure of female redclaw to males can stimulate spawning and increase reproductive efficiency. The study was conducted in dissociated (111 days) and associated (34 days) phases. Redclaw were held in vertical recirculating aquaculture systems with each system consisting of 42 individual compartments (45 cm length x 33 cm width x 25 cm height) arranged as six compartments horizontally and seven compartments vertically. The average body weight of females was 66 ± 2.2 g. In the dissociated phase, females were either kept alone (0M, n = 36) or were exposed to either 1 (1M, n = 36) or 2 (2M, n = 36) males suspended in the uppermost row of the system. During the associated phase, females (n = 108) were maintained at a sex ratio of 1M:1F. During the dissociated phase, the spawning rate was less in the control compared to the male exposed groups (2.8%, 1/36, 18.1%, 13/72 respectively; $p = 0.026$). However, the moulting rate was greater in the control compared to the male exposed groups (22.2%, 8/36; 5.6%, 4/72 respectively; $p = 0.009$). During the associated phase, there were no significant differences in spawning rate (22.2%, 8/36 and 33.3%, 24/72), mean days to spawning (21.88 ± 3.06 and 16.38 ± 2.35), moulting rate (11.1%, 4/36 and 6.9%, 5/72) and mean days to moulting (24.50 ± 3.97 and 22.40 ± 3.93) in the control and male exposed groups, respectively ($p > 0.05$). However, the mean total number of eggs (670.9 ± 26.0 and 507.0 ± 29.36), fecundity (11.00 ± 0.59 and 6.14 ± 0.47), the hatching rate (89.70 ± 1.31% and 77.50 ± 9.24%) and the total number of juveniles produced (545.70 ± 42.50 and 343.30 ± 37.47) during the associated phase were greater in the male exposed groups compared to the control group respectively; $p < 0.05$. It was concluded that pre-exposure of redclaw females to males in a dissociated recirculation system, increases spawning rate during a dissociated phase, and after exposure to males improves egg and juvenile production during an associated phase. Such a breeding strategy has potential to increase hatchery productivity.

1. Introduction

Redclaw crayfish *Cherax quadricarinatus*, is a freshwater decapod crustacean endemic to northern Australia and south-eastern Papua New Guinea (Ghanawi and Saoud, 2012; Jones, 1990; Ruscoe, 2002; Saoud et al., 2013; Webster et al., 2004). Redclaw has gained much popularity for tropical aquaculture for its outstanding attributes including robustness, simple reproductive cycle, and high market value (Jones, 1990;

Masser and Rouse, 1997; Webster et al., 2004). The domestic and international demand for redclaw is rising tremendously, yet neither conventional pond-based production methods nor relatively new hatchery technologies have been able to consistently produce commercial numbers of healthy juveniles (Rigg et al., 2021). For the industry to expand further, greater juvenile production through effective hatchery management is necessary.

Reproduction in female decapods in commercial hatcheries is

traditionally managed by manipulation of environmental stimuli such as photoperiod and temperature (Barki et al., 1997; Karplus et al., 2003), dietary supplementation (Rodríguez-González et al., 2006, 2009), and through eyestalk ablation (Palacios et al., 1999; Sagi et al., 1997; Uawisetwathana et al., 2011). Additionally, administration of hormones (Cahansky et al., 2008; Sarojini et al., 1995a; Tinikul et al., 2014; Zeng et al., 2016), RNA interference and monoclonal antibody technology (Treerattrakool et al., 2011, 2014) are also employed to achieve ovarian maturation in decapods and results from preliminary research have shown these methods as promising for enhancing reproduction in decapods. However, their practical application is so far limited by being invasive, labour intensive, and resulting in variable reproductive responses. These include asynchronous spawning, variable hatching rates and juvenile mortality (Alfaro-Montoya et al., 2019; Barki et al., 2011; Jones, 1995a, 1995b; Masser and Rouse, 1997; Okumura and Sakiyama, 2004; Stevenson et al., 2013). Moreover, the use of monoclonal antibodies and some prospective hormones such as red pigment concentrating hormones, kisseptins, GnRH and its analogues is constrained by factors such as undetermined signalling pathways, commercial unavailability, and non-optimised dosage rates (Ngernsoungnern et al., 2009; Sarojini et al., 1995b; Thongbuakaew et al., 2016; Treerattrakool et al., 2014; Zeng et al., 2016). Furthermore, the effects of hormones on the environment have not been evaluated and consumer resistance to the use of hormones in food producing animals is growing (Alfaro-Montoya et al., 2019; McEvoy, 2016). Considering these limitations, manipulation of female reproduction in a natural way is preferable as a non-invasive and sustainable strategy.

Crustaceans can exhibit spontaneous breeding where females spawn in response to chemical cues/substances such as pheromones and peptides streamed from the presence of males held in separate tanks while physically dissociated from females but still sharing a recirculated water supply system (Aiken et al., 1984; Peeters and Diter, 1994; Waddy and Aiken, 1985). In commercial hatcheries, male and female redclaw are usually kept together at a sex ratio of 1 male to 5 females while maintaining optimal water temperatures of 28 °C and a photoperiod of 14 h light and 10 h dark (Yeh and Rouse, 1994; Yeh and Rouse, 1995). Under such conditions, the females mate with males and the released eggs are fertilised by sperm attached to the sternum of females. Spontaneous spawning in the absence of males has not been reported for redclaw. However, unlike redclaw, spontaneous spawning in absence of males has been reported to occur in *Penaeus indicus* (Peeters and Diter, 1994) and in *Heptacarpus pictus* (Bauer, 1979).

Spontaneous spawning has also been reported in fish such as groupers where males and females were housed separately but had a shared water supply system. This suggests that in groupers the presence of males plays an important role in the induction of oocyte maturation and ovulation possibly via exposure to pheromones (Amagai et al., 2022; Soyano et al., 2022). Moreover, tilapia urine from males contains a pheromone that primes the female's reproductive system by increasing the production and release rates of the maturation-inducing steroid 17,20β-P (Huertas et al., 2014). Exposure of female mice and cows to male urine accelerates the onset of puberty and improves the fecundity with larger production of offspring (Mucignat-Caretta et al., 1995; Rekwot et al., 2001).

To these author's knowledge, there is no scientific study reporting spontaneous spawning of female redclaw without the direct presence of a male. However, at the Australian Crayfish Hatchery, staff have observed female redclaw to occasionally spawn when stocked in a recirculating system where males are housed separately to the females but share the same recirculating water. An advantage of being able to induce spontaneous spawning in redclaw would be to facilitate a method of providing artificial fertilisation and cryopreservation of unfertilised eggs. Moreover, the physical presence of males in some decapod species such as freshwater caridean shrimp, *Neocaridina davidi* can enhance the onset of reproductive maturity (Tropea et al., 2018). The objective of the study was to determine if pre-exposure of female redclaw to males in a dissociated, closed recirculating system could induce spawning and enhance reproductive performance. Our hypothesis was that inclusion of 1 or 2 males within the top level of a recirculating culture system will reduce the time interval to spawning and improve the reproductive performance of redclaw females.

2. Materials and methods

2.1. Experimental site and plans

The study was conducted at the Australian Crayfish Hatchery (ACH; 19° 15′ 28.656″ S, 146° 43′ 31.908″ E), located within the tropical region of northern Australia. Redclaw were housed in vertical recirculating aquaculture systems (RAS) with each system consisting of 42 individual compartments (45 cm length x 33 cm width x 25 cm height) arranged as six compartments horizontally and seven compartments vertically. The volume of each compartment, water source, and flow rate were 14 L per compartment (breeding boxes), full RAS, and full water exchanges every 10 min in each compartment, respectively. A diagrammatic arrangement of the RAS used is illustrated in Fig. 1. All crayfish had been microchipped prior to the study (Mini Microchips Australia, Merrylands NSW. ISO 11784/11785 FDX-B Microchip) which provided the identification, date, gonadal stage, moult stage, and compartment number.

Redclaw were held at 26 ± 2 °C and 14L:10D photoperiod. Water quality (temperature, pH, dissolved oxygen, general hardness, ammonia, and nitrite was monitored weekly and animals were fed a combination of Entomix (ACH proprietary feed) and commercial spirulina pellets (multiple commercial sources) once daily. Redclaw were observed daily for mortality and moulting. The average body weight of females used in this study was 66 ± 2.2 g and were classified as being within the early ovarian developmental stage (Stage I). Gonadal maturation in females was assessed using a concentrated light source as previously described in redclaw (Jones, 1990). Briefly, females were taken into a dark room and ovarian development was determined by flashing a submersible light (10,000 Lumen) on both left and right sides and the ventral part of the carapace (Jones, 1990). Females were held in compartments in the first 6 rows and 6 columns in each system. Males were held in the top row, if included, directly above the females. All crayfish were held separately unless otherwise stated. Three such recirculating systems were used one for each treatment.

2.2. Dissociated phase

During the dissociated phase (DP), females had no exposure to males (Control) or females were held separately to males with shared culture water. Females in the control group (n = 36) were held separately in the bottom six rows of an individual RAS. No males (0M) were included in this system. In the dissociated group 1 (1M), females (n = 36) were held separately in the bottom 36 containers of an individual RAS. A total of six (6) males were held in individual containers in the top row of the system. In the dissociated group 2 (2M), females (n = 36) were held separately in the bottom 36 containers of an individual RAS. A total of twelve (12) males were held in individual containers in the top row of the treatment system. The duration of the dissociated phase was 111 days.

2.3. Associated phase

On conclusion of the dissociated phase and during the associated phase (AP) males (n = 108) were added to all females (n = 108) at a sex ratio of 1M:1F in individual compartments. Males were matched with females based on female body weight, with a maximum of 20 g size disparities. Females were checked for spawning every second day and the weight of each animal was recorded every 14 days. The duration of the AP was 34 days.
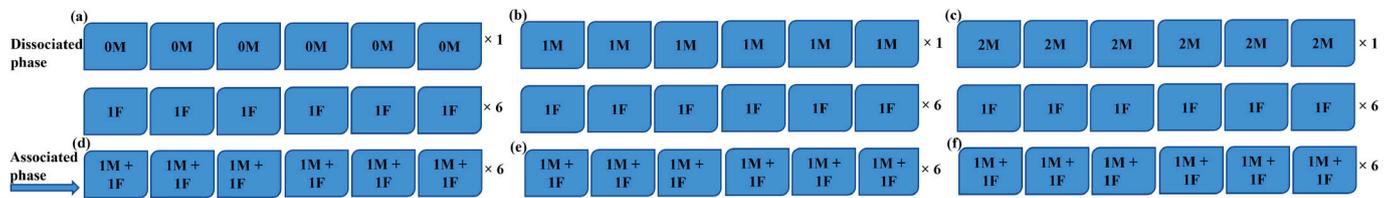
Fig. 1. Diagrammatic arrangement of the experimental procedure used for the dissociated and associated phases of the study. Dissociated phase: (*a*) 0M - females cultured in water without the presence of males (0M:6F), or (*b*) 1M - 6 males cultured with 36 females (1M:6F), or (*c*) 2M - 12 males cultured with 36 females (2M:6F) in the dissociated phase. Associated phase: (*d*), (*e*) and (*f*) each female was exposed to 1 male (1M:1F).

2.4. Determination of reproductive parameters

Spawning was assessed by retrieving females using a hand net and visually observing the ventral surface of the abdomen and opening the curled tail for the presence of eggs. Day of spawning was recorded as the day spawning was detected −1. Once spawned, the eggs were collected and counted using the 'CountThings' software (Dynamic Ventures Inc., Cupertino, CA). Eggs were collected from females using forceps and stripping eggs into a container before being photographed for counting using an automated option by the software. Fecundity was measured as eggs per g body weight of female (Rodríguez-González et al., 2006). After collection, the eggs were treated in 70% ethanol for 1 min and finally were placed in the hatching incubator (Australian Hemputin adapted from Finnish design) and monitored until they hatched. The incubator had a volume of 300 l and utilised a RAS as the water source. Hatching was identified with the complete emergence of juveniles from the eggs (García-Guerrero et al., 2003). Hatching rate was determined after initial egg collection by observing all the incubated eggs once daily and counting all the dead eggs until hatching was completed by subtracting the cumulative total number of dead eggs counted from the original number of eggs. Survival rate to the juvenile stage was measured when hatchlings moulted to become juveniles at approximately 16 days after hatching and was recorded as the (number of hatchlings – the number of surviving juveniles)/100. Craylings and juveniles were held in 1000 L tanks. From hatch to craylings no feeding was performed as they relied on their yolk sack for nutritional support. From crayling to juvenile stage of development biological floc was fed.

2.5. Data treatments and statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics version 27 for Windows (SPSS Inc., Armonk, NY, USA). A mixed effects model was used to compare the body weights of redclaw exposed to different treatments from day 0 to 145 with individual redclaw as random effects and treatment, phase, and time as the fixed effects. Proportional data were compared with a Pearson's Chi-Square test. Analysis of covariance was used to compare differences between means (days to moulting, days to spawning, eggs per female and total number of juveniles). The initial body weight of the crayfish was entered as a covariate but only retained if its effect was significant.

A log rank test, using Kaplan-Meier survival curves, was used to determine if there were differences in the survival distribution during the dissociated and associated phases for the different treatments. Cox regression was also used to assess the time to spawning in both phases in relation to treatment, initial weight and whether crayfish moulted or not. Treatment was retained in all models but variables that did not affect the dependent variable ($p > 0.10$) were removed from final models. Initial analyses treated 1M and 2M treatments (i.e., exposure to 1 and 2 males, respectively) as separate treatments. However, for every variable examined, no significant differences were found between the two treatments. Therefore, data from these two treatments were subsequently combined (Tables 1 and 2) and compared with the results from the control group.

Table 1
Reproductive characteristics of female redclaw during the dissociated phase.

Variable	Control	Exposure to males	p
n	36	72	–
Spawned (%)	2.8 (1/36)	18.1 (13/72)	0.026
Moulting (%)	22.2 (8/36)	5.6 (4/72)	0.009
Days to moulting	23.75 ± 5.66	52.75 ± 6.29	<0.001

Table 2
Reproductive characteristics of female redclaw during the associated phase.

Variable	Control	Exposure to males	p
n	36	72	–
Spawned (%)	22.2 (8/36)	33.3 (24/72)	0.233
Days to spawning	21.88 ± 3.06	16.38 ± 2.35	0.227
Eggs/female	507.0 ± 29.36	670.9 ± 26.00	< 0.001
Fecundity (eggs per g)	6.14 ± 0.47	11.00 ± 0.59	0.008
Hatching rate (%)	77.50 ± 9.24	89.70 ± 1.31	0.039
Total number of juveniles	343.30 ± 37.47	545.70 ± 42.50	<0.001
Moulting (%)	11.1 (4/36)	6.9 (5/72)	0.460
Days to moulting	24.50 ± 3.97	22.40 ± 3.93	0.722

The spawing rate in the AP was greater than in the DP for both treatments (Control DP versus AP: 2.8%, 1/36 vs 22.2%, 8/36; $\chi_1^2 = 6.22$, $p = 0.013$; exposed to males DP versus AP: 18.1%, 13/72 vs 33.3%, 24/72; $\chi_1^2 = 4.40$, $p = 0.036$).

2.6. Ethical statement

Ethics approval was not required for the conduct of this study on crustaceans by the James Cook University, Animal Ethics Committee. On completion of the study, animals were returned to the breeding colony for subsequent reuse in the breeding program.

3. Results

The initial body weight of crayfish and carapace length were highly correlated ($r^2 = 0.87$; $F_1 = 698.3$, $p < 0.001$) so only body weight was included in statistical models. Using a linear, mixed effect model no significant effect of treatment on body weight was detected (Fig. 2; $F_1 = 0.043$, $p = 0.836$), however, differences in body weight over time were detected between phases (F1 = 7.32, $p = 0.007$). Mean body weight was greater in the dissociated compared to the associated phase (65.91 ± 0.777 vs 65.59 ± 1.262, respectively) and changed over time (F1 = 7.79, $p = 0.005$). However, a phase by time interaction was, found indicating a highly significant difference in slope (rate of change in weight) between Phases (F1 = 9.87, $p = 0.002$). During the dissociated phase, the slope was marginally positive for the control group ($b_1 = 0.0024$), but slightly negative ($b_1 = -0.0003$) for the group exposed to males (Fig. 2). In the associated phase the slope was negative for both control and male exposed group ($b_1 = -0.0164$ and $b_1 = -0.0191$, respectively; Fig. 2) indicating that both treatments lost body weight during the associated phase although the difference was significant the amount weight loss was very small.
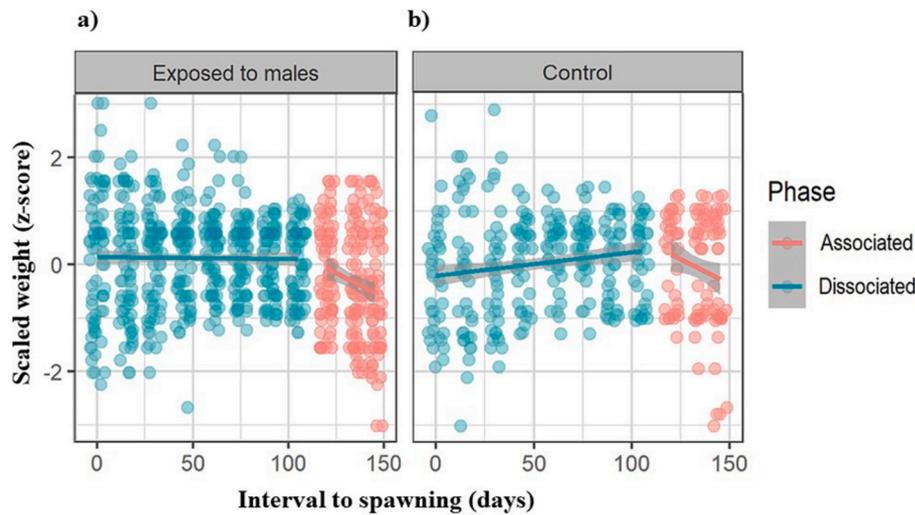
Fig. 2. Body weight (mean ± SEM) of redclaw crayfish over the duration of the study that were (a; 1M and 2M) and were not (b; Control) exposed to males.

3.1. Dissociated phase

During the DP the survival distributions for the days to spawning for the two interventions differed (Fig. 3; $\chi^2_1 = 4.93$, $p = 0.026$). Using Cox's regression time to spawning during the DP was not associated with initial body weight or moulting ($p > 0.730$) but tended to be affected by treatment ($p = 0.058$, OR: 7.13, 95% CI 0.93–54.5). During this phase the survival rate did not differ significantly between the females that were and were not exposed to males (90.3% and 86.1%, respectively; $p = 0.52$). The reproductive characteristics associated with the DP are listed in Table 1. The percentages of redclaw that spawned in the DP was significantly greater in the females exposed to males compared to the control group. The percentage of redclaw that moulted was significantly greater and the interval to moulting was significantly shorter in control group when compared with females that were exposed to males (Table 1).

3.2. Associated phase

During the AP the survival distributions for the two interventions did not differ significantly (Fig. 4; $\chi^2_1 = 1.59$, $p = 0.207$). Using Cox regression, time to spawning during the AP was not associated with
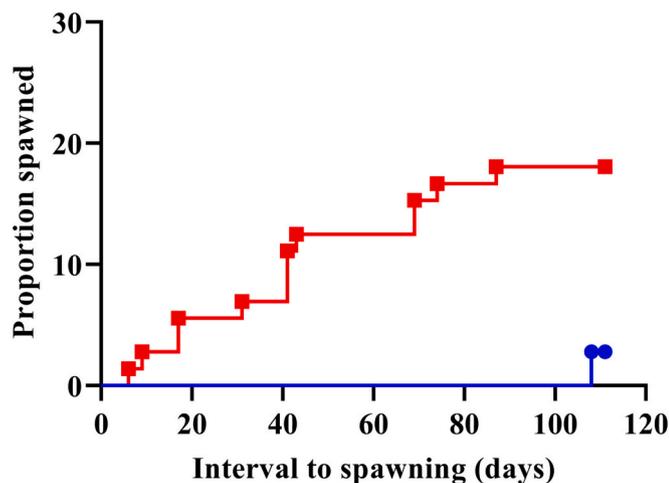

Fig. 4. Kaplan-Meier survival curve (distribution) of the proportion of redclaw crayfish spawned during the associated phase of the study for redclaw crayfish that were (■; 1M and 2M) and were not (●; Control) exposed to males.
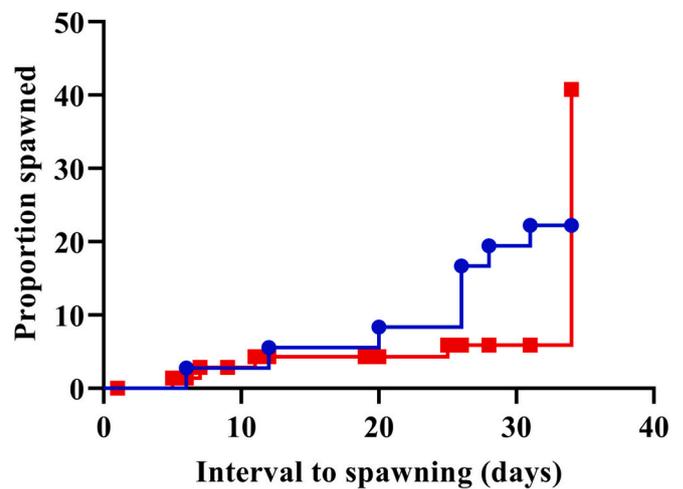
treatment ($p = 0.215$; OR $= 1.66$; 95% CI $= 0.75$–3.69), or initial weight or whether crayfish moulted or not ($p > 0.220$). The survival rate of the control and male exposed group in the AP was identical to that during the DP; hence again no significant difference was detected. Reproductive characteristics associated with the AP are listed in Table 2. The percentage of animals spawning and moulting as well as the days to spawning and moulting were not significantly different between control and the male exposed group. The total number of eggs per female, fecundity, hatching rate and total number of juveniles produced were significantly greater for the male exposed group compared to the control group (Table 2). During the AP the initial female body weight did not significantly affect the total number of eggs/female ($r^2 = -0.01$, $p = 0.41$), but it did significantly affect fecundity ($r^2 = 0.60$, $p < 0.001$; Fig. 5). There was a tendency for initial body weight to affect the total number of juveniles ($r^2 = 0.07$, $p = 0.086$) but the regression line only explained 9.5% of the variation and so poorly represented the relationship.

4. Discussion

The primary objective of the present study was to determine whether pre-exposure of females by the indirect presence of males stimulates
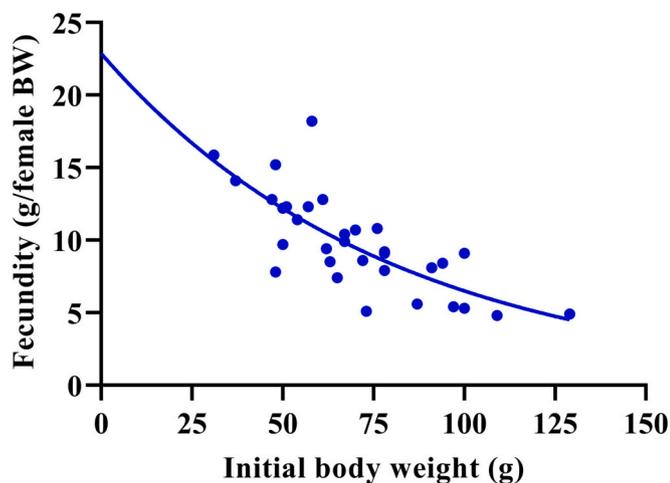

Fig. 3. Kaplan-Meier survival curve (distribution) of the proportion of redclaw crayfish spawned during the dissociated phase of the study for redclaw crayfish that were (■; 1M and 2M) and were not (●; Control) exposed to males.

Fig. 5. Quadratic relationship between the initial body weight and fecundity of redclaw crayfish.

spawning and increases reproductive performance in female redclaw. Our interpretation of the results indicates that pre-exposure of females to males in a dissociated stocking arrangement enhanced the spawning rate during a DP and significantly increased the number of eggs and juveniles produced when the females were subsequently associated directly with males. The significantly greater spawning rates in the DP and juvenile production in the AP in females subjected to pre-exposure to males was most likely induced through exteroceptive stimuli in the form of chemical cues produced by males as reported in Angler fish *Pterophyllum Scalare* (Chien, 1973). In female *P. scalare,* the visual and chemical signals from male stimulated the release of gonadotrophin hormones, which resulted in rapid development of the gonads and following ovulation and spawning compared to the group held isolated from any kind of male stimuli (Chien, 1973). This also corroborates the findings of Mozambique tilapia, where exposure of females to male urine augmented the production of the oocyte maturation hormone 17,20β-P to synchronise the spawning and fertilisation by mediating the female endocrine system (Huertas et al., 2014). Additionally, the chemosensory system plays a significant part in the coordination of aggregation and spawning behaviours in sea cucumbers *Holothuria arguinensis*, where water conditioned by males contain labile compounds such as phosphatidylcholines that attracted conspecifics and initiated spawning in both sexes (Marquet et al., 2018). Studies on crustaceans showed that the properties of urinary sex pheromones are crucial for promoting effective partner choice and mating, which raised the possibility that such chemical communication in crustaceans might trigger reproductive responses as well (Barki et al., 2011; Kamio et al., 2005). However, unlike fish, in crustaceans it is yet to be reported that chemical communication promotes spawning and egg production in females that are isolated from males.

In the present study, during the DP, the mean body weight of females in the control group increased slightly, a greater percentage moulted, and a reduced percentage spawned compared to the group indirectly exposed to males within the same recirculating system. Significant weight gain has also been achieved in other decapod crustaceans including crayfish, crabs and prawns following moulting (Cameron, 1989; Hammond et al., 2006; Xu et al., 1993). The percentage of crayfish that moulted in the AP also decreased by 50% from 22.2% during the DP to 11.1% in the AP, whereas, in the male exposed group the moulting percentage increased by a smaller percentage of 23.2% (5.6% to 6.9%) from the DP to the AP indicating that the presence of males either directly or indirectly inhibits moulting. Our results are also consistent with an earlier study on redclaw, which demonstrated that moulting took place after the breeding season when animals were reared at 26 to 28 °C temperature and 14 h light and 10 h dark condition (Barki et al.,

1997). Our findings are also in agreement with other studies conducted on *Scyonia ingentis* and *Penaeus indicus*, where moult frequency was greater in immature females in contrast to sexually mature females that spawned multiple times with no moulting (Anderson et al., 1985; Aquacop, 1975; Emmerson, 1980). Moreover, in banana shrimp *Penaeus merguiensis*, it was reported that spawning occurred before moulting and freshly moulted females did not have mature (stage III or IV) ovaries (Crocos and Kerr, 1983). Furthermore, during the non-reproductive period, in female crabs *Chasmagnathus granulate*, the percentage of crabs that moulted was greater than the percentage that spawned indicating that there was a negative association between moulting and spawning (López Greco and Rodríguez, 1999). Our results suggest that moulting and ovarian maturation are two competitive energy demanding processes which often do not occur simultaneously in decapods (Aiken and Waddy, 1980; Zmora et al., 2009). In the AP, females lost body weight in both control and male exposed groups which could be due to females partitioning and expending energy for reproduction and subsequent egg production when they were paired with males. During the AP, the spawning rate, and the mean days to spawning were not significantly different between the treatments perhaps because one-fifth (18.1%) of the animals in the male exposed group had already spawned during the DP. Insufficient time may have been available following spawning for the ovaries of these animals to mature again enabling them to spawn again during the AP which continued for only 34 days. An earlier study conducted on redclaw showed that when reared at a temperature of 25 to 26 °C and photoperiod of 12L:12D the average interval between spawning without moulting was 96 days while only 22% (6 out of 27) females spawned (King, 1993). In adult crabs *C. granulate*, the mean interval between spawning was 59.2 ± 6.7 days (López Greco and Rodríguez, 1999) which could suggest that in the present study that insufficient time was available for spawning again during the AP.

The spawning rate in the animals exposed to males indirectly during the DP was less than when they were directly exposed to them during the AP (18.1% vs 33.3%, respectively; $p = 0.036$). The exact reason for this cannot be determined from this study but could related to the longer cumulative exposure that females had to males by the end of the AP and/or the more direct contact that occurred between males and females in the AP compared to the DP. This could include exposure to behavioural and tactile cues that provided a greater stimulus for reproduction during the AP compared to the DP. It could also be that more intimate exposure favoured greater concentrations of hormones released from males in the vicinity of females. The significant increase in spawning in the unexposed females when exposed to males strongly suggests the overall importance of the presence of males to stimulate spawning in female redclaw, which has been previously demonstrated (Huertas et al., 2014; Marquet et al., 2018).

During the AP, female fecundity was found to be inversely associated with body weight, such that as fecundity declined body weight increased. This finding is concordant with other research in crayfish *Orconectes limosus* Raf., where larger females (in total length and body weight) showed significantly lower fecundity compared to the smaller females (Graczyk et al., 2019). The fecundity (11.3 eggs per g) obtained in the present study was the greatest reported to date in redclaw where fecundity has been found to vary between 8.5 and 10.31 eggs per g female (Rodríguez-González et al., 2006, 2009). In the current study, the greatest fecundity was recorded in females that ranged from 30 to 70 g. Thus, females within this weight range may be preferable for stocking in hatcheries compared to females of greater weight to maximise reproductive potential for the commercial production of eggs. The higher fecundity per unit weight of females suggest that these younger females are at the peak of their reproduction and likely to produce a greater number of eggs. Stocking smaller compared to larger female redclaw might also be beneficial economically by requiring less space and feed for maintaining broodstock.

A significant increase in hatching rate was also observed during the

AP in the male exposed group compared to the control group. Although the exact reason for this difference in hatching rate is unknown, a greater hatching rate in male exposed group can be interpreted as an induction of ovarian development in the females that has been pre-exposed to cues from the males during the DP. As a result, the females had longer time to accumulate nutrition for yolk deposition, which could have led to the production of more viable eggs with higher quality.

This study has also identified a number of areas for further study. For example, the induction of a significantly greater percentage of females to spawn that were subjected to the indirect presence of males could be a potential technique in the production of unfertilised eggs from redclaw which may then be utilised for artificial fertilisation and cryopreservation if these techniques become able to be applied in commercial aquaculture. The significant increase of spawning in the dissociated event and the greater production of juveniles in the AP suggests that chemical stimuli are released into water by males that stimulate reproduction in females. However, the identity of those chemicals and by what sensory modalities are stimulatory cues transmitted remain unknown and warrant further research. It is also crucial to determine how long the chemicals may persist for in water. For example, in sea cucumber *H. arguinensis*, when pre-spawned male water (freshly spawned water, 2 h and 4 h aged spawned water) was added to the aquaria containing either males or females, sea cucumbers spawned that received freshly spawned water and 2 h aged spawned water. However, no sea cucumber released gametes that was stimulated with 4 h aged spawned water, indicating that the spawning ingredient had degraded or evaporated (Marquet et al., 2018). Chemical cues often have a broad spectrum, and depending on the compound's polarity, molecular size, and other physicochemical characteristics, purifying each one requires a unique set of processes and tools (Kamio et al., 2022). To investigate this, mass spectrometry-based metabolomics could be employed to identify molecules or tissue specific chemicals that are present in water that can influence reproduction in females (Jjunju et al., 2020; Marquet et al., 2018). It is also unknown if there is an optimum time and duration of exposure of females to males in a dissociated aquaculture system to enhance the production of juveniles during the associated phase. In addition, the possibility of an enhanced release of the moulting hormone in redclaw could be explored considering the higher moulting rate observed in the dissociated phase.

5. Conclusions

This study is the first to investigate the effect of pre-exposure to males in a dissociated arrangement on spawning and other reproductive parameters in female redclaw and the potential for this system to increase the production of eggs and juveniles. It was found that an aquaculture system that dissociated males from females but shared the same, recirculating water increased the spawning rate and, subsequently when females were directly exposed to males, increased juvenile production in an indoor hatchery system. Production of unfertilised eggs during a dissociated stage could be a crucial component in providing unfertilised eggs for artificial fertilisation and cryopreservation, which should greatly facilitate selective breeding program. Moreover, the demonstration of greater per unit weight fecundity of smaller young females may provide opportunities to reduce space and feed requirements within commercial hatchery systems. The novel aquaculture approach for the improvement of reproduction developed here for redclaw could potentially allow the avoidance of ethical challenges and limitations associated with more invasive reproductive technologies such as eyestalk ablation, endocrine manipulation, RNA interference and monoclonal antibody technology that have been used to induce ovarian maturation in crustaceans. However, additional research is needed to identify potential chemicals in water that stimulated reproduction in redclaw.

CRediT authorship contribution statement

Nur Un Nesa: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft. **Lisa Elliott:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Chaoshu Zeng:** Supervision, Writing – review & editing. **Rhondda Jones:** Data curation, Formal analysis. **John Cavalieri:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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