Sexual selection, cognition, and life stage in male crayfish

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Abstract: The experiment selected male red swamp crayfish (Procambarus clarkii) with body weights of (5±1) g, (10±1) g, and (15±1) g. Female and male crayfish of the same size were placed at both ends of a "Y" maze as information sources for choice experiments. The results showed that: (5±1) g male crayfish had no sexual cognition; (10±1) g male crayfish exhibited preliminary sexual cognition; and (15±1) g male crayfish demonstrated mature sexual cognition. The experimental results suggest that mature male crayfish possess relatively strong aggressiveness during the non-breeding season, making them unsuitable for high-density culture.

Keywords: Procambarus clarkii, sexual selection, "Y" maze, fighting

1. Introduction

The red swamp crayfish (Procambarus clarkii) belongs to the class Crustacea, order Decapoda, family Cambaridae, and genus Procambarus [1]. It is currently the most important freshwater crayfish species for aquaculture worldwide. Due to its omnivorous diet, rapid growth rate, and strong environmental tolerance, P. clarkii can survive drought conditions without water for up to 4 months. Cervantes-Santiago et al. [2] studied the reproductive performance of P. clarkii under culture conditions and demonstrated that crayfish can effectively regenerate under controlled artificial rearing conditions.

Fighting behavior is an important ethological mechanism in the formation of animal social structures [3]. P. clarkii exhibits aggressive behavior and typically possesses strong feeding capacity, often resulting in gluttony and food competition. Many factors influence fighting intensity and outcomes, such as age [4], sex [5], social status [6], and reproductive status [7]. This study aimed to investigate the sexual selection and fighting behavior of male P. clarkii at different developmental stages in a "Y" water maze during the non-breeding season, in order to explore their sexual cognition and provide information for the culture of P. clarkii during this period.

2. Materials and Methods

2.1. Experimental materials

Experimental crayfish: The experimental crayfish were collected from the Xu yi Aquaculture Base of Jiangsu Institute of Freshwater Fisheries. Healthy, active male Procambarus clarkii with intact appendages and body weights of (5±1) g, (10±1) g, and (15±1) g were selected. The aquarium specifications were 40 cm × 30 cm × 15 cm with a water depth of 5 cm. One pellet of shrimp and crab feed was provided every other day between 17:00 and 18:00.

Experimental water: Aerated tap water (aerated for 2 days) was used. An air pump and appropriate amount of water hyacinth (Eichhornia crassipes) were added to each aquarium to ensure sufficient oxygen and regulate water quality. The water temperature was maintained at (13±2) °C, and water was changed approximately once a week.

Experimental apparatus: The main experimental apparatus was a "Y" water maze (as shown in Figure 1).

Figure 1 shows the plan view of the "Y" water maze, which consists of two choice zones and one acclimation zone. The circular areas at the ends of the choice zones had a diameter of 15 cm; the rectangular

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areas of the choice zones were 5.8 cm wide and 16 cm long; the acclimation zone was 6 cm wide and 26 cm long; the depth was 10 cm. The maze was opaque and capable of holding water.

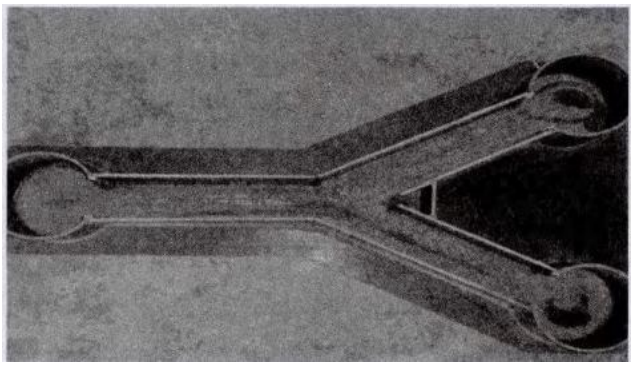

Figure 1 Physical diagram of the "Y" water maze

2.2. Experimental methods

Male Procambarus clarkii of three size classes were used: (5±1) g (42 individuals), (10±1) g (40 individuals), and (15±1) g (36 individuals), divided into three groups. Female and male crayfish of the same size were placed at both ends of the "Y" water maze as information sources. The experimental crayfish were placed in the acclimation zone of the "Y" water maze (see Figure 2) and blocked with wire mesh. Prior to each choice test, the crayfish were allowed to acclimate for 5 min. The wire mesh was then removed to allow the experimental crayfish to conduct the choice experiment. Each choice trial lasted 15 min and was conducted in darkness. An infrared video automatic recording system connected to a computer in the control room was installed above each "Y" water maze to continuously record all behaviors throughout the experiment. After the 15-min choice experiment, the information source crayfish and experimental crayfish were gently removed and returned to their original individually reared aquariums. The water in the maze was drained, and the maze was thoroughly cleaned before conducting the next choice experiment.

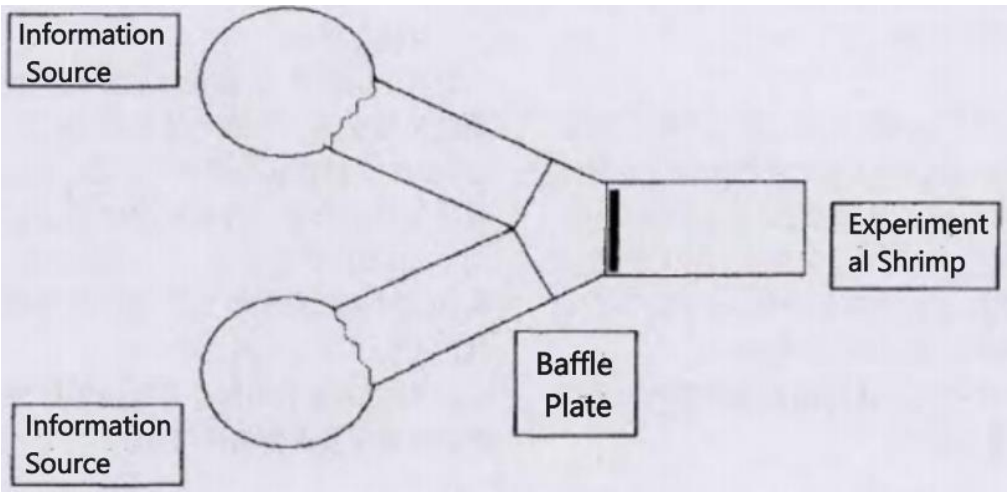

Figure 2 Schematic diagram of the "Y" water maze

2.3. Rearing management

During the experimental period, the experimental crayfish were reared in aquariums with specifications of 40 cm × 30 cm × 15 cm and a water depth of 5 cm. One pellet of shrimp and crab feed was provided every other day from 17:00 to 18:00. The water temperature was controlled at (13±2) °C.

2.4. Experimental data

2.4.1. First choice

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The time taken by experimental crayfish to choose different information sources was recorded as the criterion for first choice, measured in seconds.

2.4.2. Choice frequency

The number of times experimental crayfish chose different information sources during the choice process was recorded.

2.4.3. Choice time

The time spent by experimental crayfish choosing different information sources was recorded separately, measured in seconds.

2.4.4. Outing time and frequency

The total time and total number of outings for choice by experimental crayfish were recorded.

2.4.5. Fighting behavior

Based on the time spent by experimental crayfish choosing different information sources (male and female), the crayfish were divided into three groups: male-choosing, female-choosing, and non-choosing. Within each size class, male-choosing crayfish were divided into groups A and B, and female-choosing crayfish were designated as group C, with equal numbers in each group. Group A was paired with groups B and C for fighting experiments, and the results were observed.

2.5. Statistical Analysis

Data were analyzed using SPSS 18.0, and graphs were produced using Excel software. Binomial distribution tests were performed for first choice, and paired tests were conducted for choice and outing frequency and time. The significance level for difference tests was set at 0.05, and the extremely significant level was set at 0.01.

3. Results and Analysis

3.1. First choice of male crayfish at different developmental stages

See Table 1 for details. Most crayfish chose to move out, indicating that this batch of crayfish had strong activity and was suitable for this experiment. During the non-breeding season, at a water temperature of (13±2) °C, the three different size classes of male crayfish showed no significant preference for choosing males or females in their first choice ($P > 0.05$), selecting randomly between sexes. However, during the non-breeding season, as the body weight of crayfish increased, the proportion of choosing males gradually increased.

Table 1 First Choice

Group	Total n	No Choice	Male Chosen	Female Chosen	No Choice/Total	Male: Female	P (Female-Male)
5g	42	5	17	20	11.90%	0.85	0.743
10g	40	3	20	17	7.50%	1.18	0.743
15g	36	3	19	14	8.30%	1.36	0.608

3.2. Choice frequency of male crayfish at different developmental stages

See Table 2 for details. During the non-breeding season, at a water temperature of (13±2) °C, the three different size classes of male crayfish showed no significant difference in the frequency of choosing females and males ($P > 0.05$). However, during the non-breeding season, as the body weight of crayfish increased, the frequency of choosing males and females by the same size class of male crayfish increased, indicating that as the body weight of male crayfish increased, their gonadal development became relatively more mature, and their selectivity and activity gradually increased.

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Table 2 Choice Frequency

Frequency (mean± SD)/times			P (Female-Male)
Group	Male	Female	
5g(n=37)	1.57±1.24	1.16±1.07	0.214
10g(n=37)	1.70±1.27	1.41±0.98	0.254
15g(n=33)	2.09±1.40	1.82±1.36	0.444

3.3. Choice time of male crayfish at different developmental stages

See Table 3 for details. During the non-breeding season, at a water temperature of (13±2) °C, the three different size classes of male crayfish showed no significant difference in time spent choosing females and males ($P > 0.05$). However, during the non-breeding season, at a water temperature of (13±2) °C, the same size class of male crayfish spent more time choosing males than females, indicating that male Procambarus clarkii of the same size class were more likely to choose males than females during the non-breeding season at (13±2) °C.

Table 3 Choice Time

Time (mean± SD)/s			P (Female-Male)
Group	Male	Female	
5g(n=37)	293.11±264.16	246.62±301.60	0.597
10g(n=37)	252.70±229.58	155.05±205.95	0.125
15g(n=33)	215.30±184.24	168.79±199.00	0.441

3.4. Outing time and frequency of male crayfish at the same developmental stage

See Table 4 for details. During the non-breeding season, at a water temperature of (13±2) °C, there was no significant difference in outing frequency between 5 g and 10 g male Procambarus clarkii ($P = 0.608$). The outing frequency of 15 g male crayfish was extremely significantly greater than that of 5 g male crayfish ($P = 0.010$), but showed no significant difference from 10 g male crayfish, indicating that as the body weight of male crayfish increased, their gonads became relatively more mature and their activity gradually increased. Regarding choice time, 5 g male crayfish spent significantly more time choosing than 10 g male crayfish ($P = 0.025$), and extremely significantly more time than 15 g male crayfish ($P = 0.003$). There was no significant difference in choice time between 10 g and 15 g crayfish ($P = 0.942$). Based on the results of choice frequency and outing time, 5 g crayfish spent the longest time per choice, while 15 g crayfish spent relatively shorter time per choice.

Table 4 Comparison of Outing Time and Frequency

Group	Frequency (mean± SD)/times	P(5~10g)	P(10~15g)	P(5~15g)
5g(n=37)	2.73±1.24			
10(n=37)	3.11±1.65	0.608	0.179	0.010
15g(n=33)	3.91±1.88			
5g(n=37)	539.73±201.77			
10g(n=37)	407.76±217.47	0.025	0.942	0.003
15g(n=33)	384.09±172.28			

3.5. Fighting behavior of male crayfish at different developmental stages

See Table 5 for details. The data indicate that during the non-breeding season, fighting behavior was similar between groups A and C of the same size class. Male crayfish that spent more time choosing males

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(groups A and C) showed stronger aggressiveness and were more robust than those that spent more time choosing females (group B), as indicated by A+ total/A- total ratios all greater than 1.

Table 5 Fighting Statistics of Groups A, B, and C of the Same Size Class

Group	5g	10g	15g
A-B group	23-	14-	17-
	27+	20+	24+
A-C group	25-	16-	22-
	25+	18+	19+
A- (AB group)/A-total	0.479	0.467	0.436
A- (AC group)/A-total	0.521	0.533	0.564
A+ total/A-total	1.08	1.27	1.10

4. Discussion

Schneider et al. [9] studied chemical signaling in the establishment of social dominance in Procambarus clarkii and indicated that crayfish require a familiarization process when entering a new environment. The experimental results showed that during the non-breeding season, at a water temperature of (13±2) °C, male P. clarkii of three size classes— (5±1) g, (10±1) g, and (15±1) g—showed no significant difference in their first choice between males and females, selecting randomly. However, during the non-breeding season, as the body weight of crayfish increased, the ratio of choosing males to females gradually increased. It can be inferred that during the non-breeding season, at (13±2) °C, as the body weight of crayfish increases, their sexual awareness gradually strengthens, and male crayfish are more likely to choose other males.

The results showed that during the non-breeding season, male P. clarkii of (5±1) g, (10±1) g, and (15±1) g showed no significant difference in the frequency of choosing females and males. From outing time and choice frequency, it can be seen that as the body weight of crayfish increased, the frequency of choosing males and females and outing time increased, indicating that the activity of crayfish between (5±1) g and (15±1) g increases with body weight.

The experimental results showed that during the non-breeding season, male P. clarkii that chose males were more aggressive and robust than those that chose females. P. clarkii has a strict dominance hierarchy, and individuals fight when competing for status, food priority, and space ownership within the population. Tang et al. [10] studied the selectivity of P. clarkii for several artificial shelters and mentioned that crayfish are highly territorial; when multiple crowded crayfish enter each other's territories, fighting occurs, leading to death. Combined with the results of this experiment, it can be inferred that high-density culture is not suitable for P. clarkii during the non-breeding season.

Gong et al. [11] mentioned in their study on the reproductive biology of P. clarkii that through year-round sampling analysis, the sexual maturity age of P. clarkii is about 1 year, with a minimum body length of 7.1 cm and minimum body weight of 20 g for males. In summary, (5±1) g male P. clarkii basically have no sexual cognition, (10±1) g male P. clarkii have preliminary sexual cognition, and (15±1) g male P. clarkii have mature sexual cognition. The experimental results suggest that mature male P. clarkii still maintain strong aggressiveness during the non-breeding season and are unsuitable for high-density rearing. In the production culture of male P. clarkii during the non-breeding season, attention should be paid to stocking density to avoid high-density culture and reduce casualties caused by fighting.

5. Conclusion

This study systematically investigated the sexual cognition characteristics and behavioral performance of male Procambarus clarkii at different developmental stages during the non-breeding season using a "Y" maze choice experiment. The results demonstrated that as body weight increased from (5±1) g to (15±1) g, the sexual cognition ability of male P. clarkii showed a clear developmental gradient: (5±1) g individuals

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basically lacked sexual cognition, (10±1) g individuals began to exhibit preliminary sexual cognition, and (15±1) g individuals achieved mature sexual cognition. Notably, sexually mature individuals still displayed strong aggressive tendencies and fighting behavior during the non-breeding season, which is closely related to the strict dominance hierarchy of P. clarkii.

The findings of this study have important practical implications for the scientific culture of P. clarkii. Given that mature male individuals maintain high aggressiveness and territorial behavior during the non-breeding season, it is recommended that stocking density be reasonably regulated according to crayfish size in production culture to avoid fighting casualties caused by spatial competition, thereby improving survival rates and economic benefits. Future research could further explore the influence mechanisms of environmental factors such as temperature and light on the social behavior of P. clarkii during the non-breeding season, providing theoretical support for improving year-round culture management techniques.

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