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Some fecundity parameters and ovarian maturity criteria of ornamental red cherry shrimp (*Neocaridina davidi*)

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Abstract: The red cherry shrimp (RCS) *Neocaridina davidi* is a tiny shrimp that has a bright red color; therefore, it has become a popular ornamental species. The aims of this study are to determine the fecundity, egg size, and maturation time of the RCS at different sizes and also to determine its ovarian maturity criteria. A total of 18 adult female shrimps in 3 different size groups based on carapace length were used. They were grouped as small (S: 1.5–1.7 cm), medium (M: 1.8–2.0 cm), and large (L: 2.1–2.3 cm); a total of 36 adult male shrimps (1.5 ± 0.2 cm) were used in the research. The parameters observed in this study were fecundity, size of the egg (diameter), and gonadal maturity of the female. The L-size group of the RCS obtained the highest fecundity, the highest uniformity of egg size, and the fastest maturation period, so it can be concluded that the best reproductive performance was observed in the L-size group of RCS. We also determined 4 ovarian maturity criteria of the RCS so that they can be used for production efficiency, in further research, and for its culture.

Key words: Fecundity, egg size, maturation time, maturation stages

1. Introduction

The genus *Neocaridina* consists of freshwater shrimp species that can be found naturally in China, Japan, Korea, Vietnam, and Taiwan [1]. Red cherry shrimp (RCS) *Neocaridina davidi* [2] belongs to this genus, and it is a tiny shrimp with a bright red color. Therefore, it has become an important aquarium species in many European countries [3]. In Indonesia, RCS culture has become increasingly popular as a freshwater ornamental shrimp and, because of this, it is currently being exported [4]. However, basic information about the reproduction biology of this species is still rare in the literature [5].

The reproductive performance of shrimp is influenced by many factors including the size of the broodstock and its habitat [6], cultivation systems and environmental conditions [2,7–9], season and age [10], diet [11,12], and genetics [13]. Studies on some of the factors that influence the reproductive performance of RCS have been carried out, including those affecting diet [14,15] and temperature [16,17].

Although the reproduction performance of RCS is an important restrictive factor for the successful mass production of seeds in RCS culture [18], there are very few studies related to this area of research. RCS commercial seed production is commonly practiced under captive breeding conditions through the propagation of domesticated broodstock [14] in southern Asian countries. Reproductive performance parameters that determine the capacity of the seed production of female broodstock are fecundity and maturation periods. On the other hand, the size of the egg also potentially affects the quality of the seed [19]. For the successful gonadal investigations in RCS, the criteria of ovarian maturity should be known to measure the maturation periods. As an example, the rapid determination in the examination of ovarian maturity and ovary development criteria of the deep-water shrimp (*Aristaeomorpha foliacea*) has been previously studied [20,21].

Information about the optimal broodstock size needed to produce seeds is very important for the efficiency at the

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hatchery and increases the quality of the seeds produced in aquaculture. The question is whether larger females are able to produce more and better-quality seeds. The allocation of energy divided for growth and reproduction in proportion depends on body size. Most of the energy in the small female is used for growth, but it is mostly used for reproduction in large females [22–24]. The relationship between size and reproductive performance in RCS is unknown, and we aimed to determine the fecundity, egg diameter, and maturation period of female RCS at different sizes, along with the criteria of ovarian maturity.

2. Materials and methods

2.1. Origin and culture of the shrimp

This study was conducted at the laboratory of the Study Program of Aquaculture, Banyuwangi Campus, Faculty of Fisheries and Marine at Universitas Airlangga, Banyuwangi in East Java, Indonesia between February and March 2018. A total of 18 female and 36 male adult RCSs that were around 5 months old were collected for this study from a commercial RCS hatchery (Aquavirus Fish Farm Co., Indonesia). The females were divided into 3 different size groups based on carapace length: small (S: 1.5–1.7 cm), medium (M: 1.8–2.0 cm), and large (L: 2.1–2.3 cm); the size of the males was 1.5 ± 0.2 cm. They were all kept in terms of their size groups with a ratio of 1:2 in 3-L plastic containers. Gentle aeration in the water was initiated, gravel was placed at the bottom, and the aquatic plant *Cabomba caroliniana* was added to the containers. The shrimps were fed with commercial shrimp feed (Fengli, Matahari Sakti Ltd., Indonesia) and spinach leaves boiled for about 5 min (2 g per day in the morning). Half of the water in the containers was replaced every 5 days. The shrimps were kept until they produced fertile eggs for fecundity analysis and egg diameter measurement. After the first examination, they were kept for a second period of maturation in order to determine maturation stages. The water temperature was measured at 28–29 °C, and the dissolved oxygen level was measured at a minimum of 5 ppm.

2.2. Parameters analysis

After the first maturation, the eggs attached in the pleopods of 6 individuals from each size group were carefully collected in distilled water filled in different petri dishes using an inoculation needle. They were then counted one by one using a hand counter under a stereo microscope (Nikon, Japan). The diameters of eggs from 3 size groups were measured using a compound Eclipse E200-LED light microscope connected to a video monitor (40×) with NIS Elements Imaging Software (Nikon, Japan). The diameters of the ovoid shape egg was calculated with the formula $\sqrt{(Dxd)}$, where D represents the larger section and d represents the smaller section of the egg [25].

The same shrimps were kept in the same conditions for second maturation to determine the ovary maturation criteria. Visual observation of ovary maturation was done by checking the appearance of the ovary through the carapace under a stereo microscope (Nikon, Japan) connected to a camera; the days of maturity stages were also recorded.

To measure oocyte diameter, ovary tissues (N = 3 in each maturity stage) were prepared and stained with hematoxylin-eosin [20]. The diameter of 10–15 oocytes of each specimen was measured under a light microscope (40×) with NIS Elements Imaging Software (Nikon, Japan), and the ovoid shape formula mentioned above was used and the days of maturity stage were recorded.

The observed parameters were analyzed descriptively and statistically. Statistical analysis was carried out using variance analysis (one-way ANOVA) with a 95% confidence level. Significance between different size groups of shrimps was identified using Duncan's test with SPSS version 7.0. statistical software.

3. Results

The size difference in RCS has a significant effect on the fecundity and maturation period ($P < 0.05$). The highest fecundity was obtained in the L group (53.16 ± 4.26 eggs per individual), followed by the M group (42.66 ± 8.23 eggs per individual), and the S group (22.00 ± 4.04 eggs per individual); each of them was significantly different ($P < 0.05$). This shows that fecundity is directly proportional to body size in RCS (Figure 1).

However, no significant differences were observed in the egg diameters among the 3 groups ($P > 0.05$). Egg diameter was recorded as lower in the L group (859.70 ± 3.42 µm) based on standard deviation. Egg diameter was recorded as 914.74 ± 60.03 µm and 882.09 ± 100.41 µm in the S group and M groups, respectively (Figure 2).

The maturation period tended to be shorter in the L group (29.00 ± 0.89 days), and this was significantly different ($P < 0.05$) from the S group (30.00 ± 0.63 days). However, the S and M groups were not significantly different ($P > 0.05$) (Figure 3).

Based on specimens examined for ovary maturation stage observation, a slight difference in the color was observed in RCS, but the condition of the ovaries varied from being transparent, small, and duct-like while later developing from the frontal body as darkened, swollen and subdivided into distinct lobes. However, 4 distinct ovarian stages could be recognized by the marked differences in the color, shape, and size of the ovaries (Table 1). Below are the characteristics of these 4 ovarian stages in terms of their general appearance, cell histology, and also the duration of maturation (days).

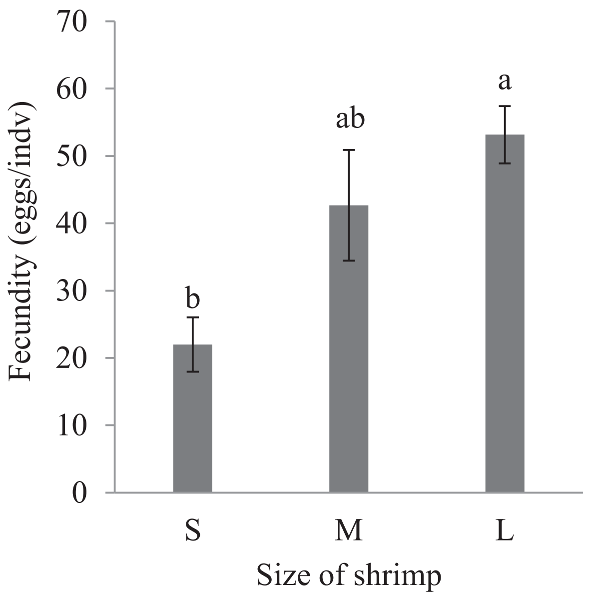

Figure 1. Fecundity of red cherry shrimp (*Neocaridina davidi*) at different sizes (N = 6, mean ± SD). Different letters above the bars show a significant difference (P < 0.05).

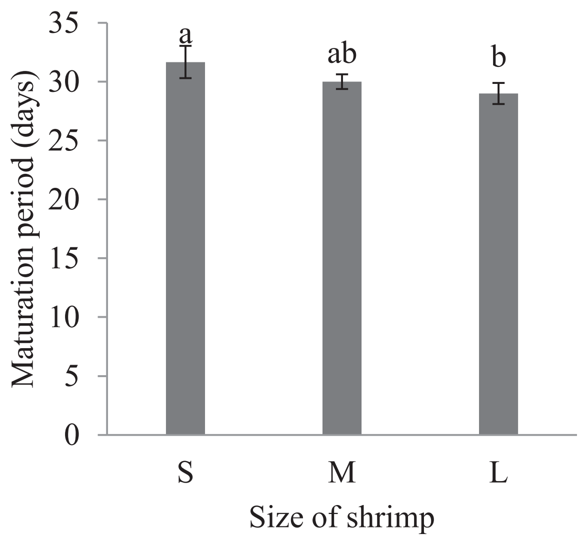

Figure 3. Maturation period of red cherry shrimp (*Neocaridina davidi*) at different sizes (N = 6, mean ± SD). Different letters above the bars show a significant difference (P < 0.05).

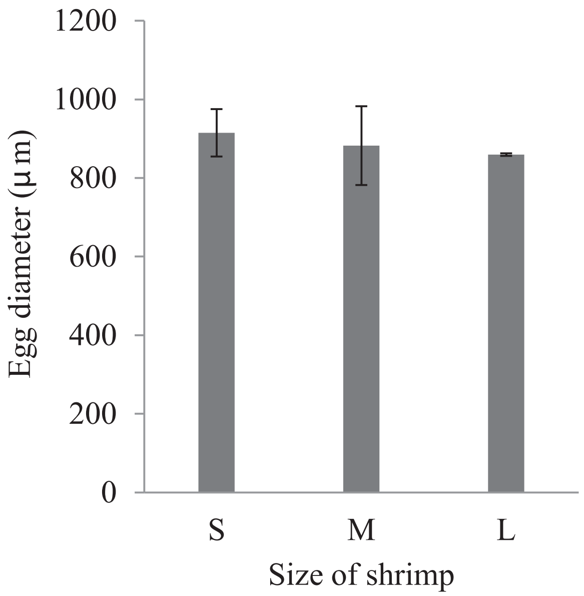

Figure 2. Egg diameter of red cherry shrimp (*Neocaridina davidi*) at different sizes (N = 6, mean ± SD).

In the 1st determined stage (Figure 4a) the frontal and lateral lobes were thin and clearly separated and greenish brown. The caudal lobes could be seen as a tiny transparent duct through the alimentary canal. The 1st stage ovary occurred on the 1st day after spawning and oocyte diameter ranged between 6.25 and 143.21 mm (Table 1).

During the 2nd determined stage of the ovary (Figure 4b), the frontal lobe became enlarged and thicker caudal lobes were observed. Tiny transparent ducts through the alimentary canal could still also be seen. The color of the ovary was darker than during the 1st stage. This stage occurred on the 14th day after spawning and oocyte diameter ranged from 48.39 to 297.40 mm (Table 1).

The ovaries in the 3rd determined stage (Figure 4c) expanded and subdivided into 2 distinct lobes. The caudal lobe enlarged so that it was more visible. The 3rd stage ovary occurred between the 20 and 24th days after spawning and oocyte diameter was 134.95–374.80 mm (Table 1).

The ripe ovary was seen in the 4th determined stage (Figure 4d), and it was characteristic of a thickened lateral lobe. Meanwhile, the caudal lobe expanded into 3 lobes; the last lobe got smaller and closer to the alimentary canal. The 3rd stage ovary occurred on the 28–33rd days after spawning and oocyte diameter ranged from 249.44 to 500.00 mm (Table 1).

4. Discussion

In this study, the larger female of RCS obtained the highest reproductive performance in fecundity and maturation period and the lowest variation in egg size. Previously, similar results in fecundity were also obtained in studies of *Penaeus paulensis* [6], *Philocheras trispinosus* [26], *Farfantepenaeus paulensis* [27], red front shrimp (*Caridina gracilirostris*) [28], and rose shrimp (*Parapenaeus longilostris*) [28]. Fecundity increased proportionally in size, and the relationship between size and weight

Table 1. Different stages of ovary condition in the red cherry shrimp (*Neocaridina davidi*) according to histological and visual investigation; (das: day after spawning).

Ovary	Maturation time (days)	Appearance through the carapace	Oocyte diameter (µm)
Stage I	1st	Ovary is thin, translucent greenish brown and has a nebulous appearance.	6.25–143.21
Stage II	14th	The color of the ovary becomes thicker and turns a darker color. It enlarges and moves into the alimentary canal and covers nearly the entire carapace.	48.39–297.40
Stage III	20–24th	Ovary increases in size becoming longer until half of the body is in the frontal and brown color.	134.95–374.80
Stage IV	28–33rd	Ovary expands until swollen and covers almost the entire cephalothorax and enlarges until more than half of the body is in the frontal area. The color is now dark brown.	249.44–500.00

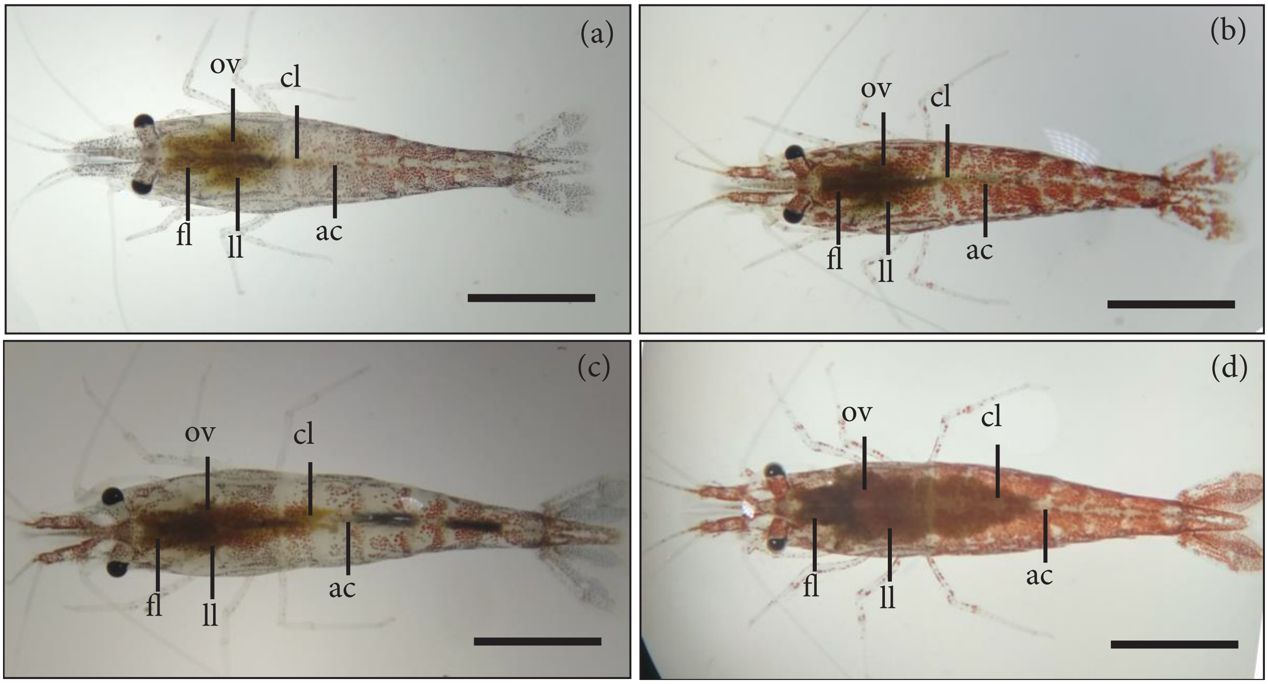

Figure 4. Different maturity stages of red cherry shrimp (*Neocaridia davidi*) from dorsal view: ov = ovary; fl = frontal lobe of ovary; ll = lateral lobe of ovary; cl = caudal lobe of ovary; ac = alimentary canal. First ovary stage (a); second ovary stage (b); third ovary stage (c); fourth ovary stage (d). Bar: 0.5 cm.

with fecundity was parallel with linear function [26,28]. The size of pleopods in relation to body size can be important to support fecundity [26]. The eggs of RCS are attached to pleopods. The large size of these organisms is associated with body size, and these large pleopods can accommodate many eggs, which could affect the number of eggs produced. To ensure the relationship between the size of pleopods and fecundity in RCS, further research should be carried out.

Egg size has an effect on the subsequent development in the early life cycle, which affects offspring fitness [19] and also affects the survival rate of fry [29,30]. In this study, the egg size of the L group was smaller than the others; in addition, a low variation in egg size was found. A previous study of freshwater prawn (*Macrobrachium lamarrei*) shows that egg size decreases with an increase in body weight [31]. Information about the relationship between body size and egg size in shrimps is lacking; however, their correlations are different for each species, and how this occurs has not yet been explained. The correlations between egg size and female size reflect the effects of directional selection on egg size [19]. The variation in egg size is related to energy content because large eggs have more yolk [32] and a longer hatching time. Our results showed a low distribution in the L group, which indicates that the energy from the yolk was spread out evenly on each egg. Egg size is also directly proportional to larvae survival in brown trout (*Salmo trutta* L.), and similar results were obtained in other species [29].

In line with fecundity and egg size, the increase in maturation time was obtained in the L group and was approximately 1 and 1.5 days faster than the other 2 size groups, M and S, respectively (Figure 3). Similarly, the large female of *Penaeus monodon* rematured and spawned more frequently than the small one [33]. The faster period of gonadal maturation in the L group shows that the results in this study support the theory: most of the energy in the smaller female is used for growth while the larger female mostly used energy for reproduction [22–24].

The stages of or criteria for ovarian maturity in female RCS were reported for the first time in this study. The criteria for ovarian maturity in the study of some shrimp species were classified into 5 stages in the spotted pink shrimp (*Penaeus brasiliensi*) [34], the deep-sea sponge-associated shrimp (*Spongicola japonica* Kubo) [35] and classified into 6 stages in some penaeid shrimps from the coast of Pakistan (36). We classified the criteria of RCS ovarian maturity into 4 stages, which were similar to the stages of ovarian maturity in deep-water shrimp (*Aristaemorpha foliacea*) [20,21]. The ovarian maturity stage of female RCS determined was based on the size and number of lobes of the ovary seen through the carapace. The further stages moved up, the more the number and size of lobes increased. Various species of shrimps also show increased size and number of lobes in the ovary along with maturity [20,21,34–36]. Based on the color observation of the ovary, there was a slight change in its color while the maturity stages increased. This was different from other shrimps in which there was a significant color change as the ovarian maturity stage progressed. For example, ovary color changes from translucent and colorless to pale black in the deep-water shrimp (*Aristaemorpha foliacea*) [20], and ovary color changes from white translucent to dark green in the sea-bob-shrimp (*Xiphopenaeus kryoyeri*) [37]. This shows that each shrimp species has different ovarian maturity characteristics, especially in terms of color.

Increased oocyte diameter in the ovary maturation is caused by the accumulation of vitellogenin from the process of vitellogenesis because it is known that vitellogenin will eventually be used by the embryo as an energy reserve to grow and develop [38,39].

The L-size group of RCS obtained the highest fecundity, the most similar egg size, and the fastest maturation period; therefore, it can be concluded that RCS larger than 2.0 cm in carapace length were found to exhibit the best reproductive performance. Ovarian maturity criteria in the RCS are also explained, which is important information for RCS culture and also for future studies. It can be suggested to producers of RCS to check female maturity every 30 days at a minimum after spawning for less stress condition. The relationship between reproductive performance and the sizes of the shrimp, as well as the criteria for ovarian maturity determination reported here, were not unexpected; however, these results may be useful for researchers who study the reproduction of RCS or for comparative studies on the reproduction of other shrimps/crustaceans.

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Conflict of interest

The authors declare that there is no conflict of interest in this research.

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