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Breeding and Life Cycle of *Neocaridina denticulata sinensis* (Kemp, 1918)

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ABSTRACT

Neocaridina denticulata sinensis (Kemp, 1918) or also known as red cherry shrimp (RCS) became popular in aquarium industry since its first introduction in 2003 until now. Little is known of this particular species since documented report is very scarce. Thus, this study was conducted to gather some information on its breeding behavior and life cycle until first maturity in laboratory condition. Fertilized eggs were found to be oval in shape with color varies from greenish to yellowish. The egg size was comparatively large, with an average diameter of 1.19 mm. Embryonic development of *N. d. sinensis* lasted for 15 days at 27°C. Newly hatched larvae look like a miniature version of the adult with an average total length of 2.3 mm. A female can produced about 21-51 larvae per hatching. Larger females produced more larvae. It takes 60 days for larvae to reach juvenile stage, where male and female were still undifferentiated. Juveniles become adults 15 days later. Females were obvious with the presence of orange colored ovary at the cephalothorax region. Within one to three days, these males and females are ready to spawn. During this study, *N. d. sinensis* cultured in freshwater were found to be susceptible to clitellate annelid (*Holtodrilus* sp.). Culture of this species in slightly saline condition between 5-10 ppt was an effective treatment for this annelid.

Key words: *Neocaridina denticulata sinensis*, breeding, first maturity, life cycle

INTRODUCTION

Neocaridina denticulata sinensis is an indigenous species of Japan, Korea, China, Vietnam and Taiwan (Hung *et al*., 1993). According to Cai (1996), *N. d. sinensis* comes from pond, river, agricultural canals, mountain streams and reservoirs of its indigenous area. Previously *N. d. sinensis* from Nu'uanu Stream, O'ahu was incorrectly identified as *Caridina weberi* by Devick (1991). Unlike the native freshwater atyid shrimp *Atyoida bisulcata*, *N. d. sinensis* does not have an obligate marine phase (Hung *et al*., 1993). As they occupy similar habitats, it is possible that *N. d. sinensis* can compete with *A. bisulcata* (Englund and Cai, 1999). Holthuis (1993) stated that *N. d. sinensis* was placed under the genus *Caridina* before being reclassified under *Neocaridina*. This classification was based on the morphological difference in male, that is the presence of endopod on the first pleopod. While, Kubo (1938) strengthen it with the appearance of appendix masculina on its second pleopod.

Many freshwater shrimp from the family Atyidae have been growing in popularity in the aquarium industry over the past years (Heerbrandt and Lin, 2006). *N. d. sinensis* is one of these atyids. It was first introduced in the aquarium industry in 2003. The bright red colored shrimp looks great in contrast to green aquatic plant or against dark colored background. Not only this shrimp enriched the look of the freshwater tank, it is also excellent maintenance crews in a cleaning the aquatic plants compared to other shrimp, including the popular Amano shrimp, *Caridina japonica* (Demas, 2007). According to Niwa and Ohtaka (2006), live *N. d. sinensis* imported to Japan from Korea and China were to fulfill demands in recreational fishing, used as baits for black sea bream, *Acanthopagrus schlegeli* and black rockfish, *Sebastes inermis*. Freshwater shrimp in Kalimantan, Indonesia were sampled using light trap (Ahmadi, 2012).

In Taiwan, there are 15 species of atyid from 3 genera which include *Atyopsis*, *Caridina* and *Neocaridina* (Hung *et al*., 1993; Shih and Cai, 2007). Similar to palaemonid shrimps, atyidae demonstrated three types of larval development, normal, abbreviated and completely suppressed (Shokita, 1981; Shy, 1994). Most of these atyids undergoes the normal type of larval development and produce relatively small-sized eggs with long planktonic phase. As for abbreviated type, larval of planktonic phase only takes few days. However, for the completely suppressed type, eggs were relatively large-sized without planktonic phase since the larvae hatched in benthic form.

Successful breeding and mass production of this shrimp can provide avenue for the aquarium industry and contribute to national income. Currently an adult size *N. d. sinensis* is priced between MYR 2-3 each. Previous study by Yang and Ko (2003) provides little information on the larval development of this shrimp and none on the breeding and complete life cycle. Thus, this study was conducted with the objective to breed and observed the life cycle of *N. d. sinensis* in laboratory condition.

MATERIALS AND METHODS

Experimental works on *N. d. sinensis* were conducted in the Endocrinology Laboratory, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Tank preparation and maintenance: Different sizes of plastic aquarium tank were used for conditioning female broodstock (40×24×30 cm), conditioning male broodstock (35×17×25 cm), breeding (40×24×30 cm), separating ovigerous female (7×7×10 cm), conditioning larvae (26×16×16 cm) and life cycle observation (40×24×30 cm). Throughout this study, PVC pipe (Ø 16 mm) was used as shelter during molting. Aerated tap water was de-chlorinated and used as source of water. Aeration was provided throughout the study period.

Feeding and water quality: Broodstock and larvae were fed *ad libitum* with commercial shrimp feed containing 40% protein twice a day at 08:00 and 17:00 h. About 50% water change was carried out once a week. Few main water quality parameters measured in this study. The kits or apparatus used were; DO meter (dissolved oxygen and temperature) and API aquarium freshwater test kit (pH, ammonia, nitrite). Measurements were taken twice a week.

Broodstock conditioning: Matured males and females *N. d. sinensis* were bought from nearby aquarium shops. About 30 females and 30 males used were with average total length of 2.5±0.5 cm and placed into two separated conditioning tanks. Conditioning process takes about one month. Only females with saddle and healthy males were used.

Breeding: Ten pairs of matured male and female *N. d. sinensis* were placed in breeding tank. Observation was carried until female shrimp become ovigerous.

Larvae: Each of ovigerous females were weighed and moved to individual tank. Female was removed after the larvae hatched. Number of larvae produced by each female was counted and recorded. Larvae produced from the earlier experiment were placed and conditions in tanks for a week. After a week, only healthy larvae were used for the next experiment.

Life cycle: Thirty larvae (one week old) were placed in tank. Observation was carried out until all the larvae reached first maturity. Life cycle of *N. d. sinensis* was presented into illustration form. Total length (TL) of *N. d. sinensis* was measured from rostral tip to posteromedian margin of the telson, excluding posterior setae following Yang and Ko (2003). Larvae and eggs were observed and measured using ocular micrometer placed in the microscope eyepiece of Leica DM750, compound microscope with scale bar. Photographs were taken using Canon S95 digital camera. Ranges of water quality parameters observed throughout this study were recorded.

RESULTS

N. d. sinensis reached first maturity at around 75 days old with TL 2.3±0.2 cm. Upon reaching maturity, the presence of orange colored ovaries can be seen through the cephalothorax region of the females (Fig. 1). Appendix masculina on the second pleopod (Fig. 2) were observed in males. Mating process usually take place 1-3 day(s) after the introduction of male and female into the same tank. Female molted prior to mating.

Both molting and mating occurred rapidly in less than 10 sec. During mating process male and female of *N. d. sinensis* will be facing each other and sperm were deposited into the genital opening of female *N. d. sinensis* using its appendix masculina. The eggs were fertilized when it passed through the sperm on the way to the brood pouch. The pouch was formed by pleopods and overhanging of pleura of the female. A day after mating, female was observed to carry fertilized eggs. The eggs were incubated in the brood pouch until hatch.

Fertilized eggs were oval in shape, vary from greenish to yellowish in color. The egg size was comparatively large, with an average length 1.19 mm. Thin ribbon-like filament binds the eggs in grape-like bunches and attached it to the female's pleopod. The membrane can hardly be distinguished since it is thin, transparent and lying close to the surface.

Fig. 1: Matured female *N. d. sinensis* with visible orange colored ovaries (arrow) in the cephalothorax region

At 27°C, embryonic development of *N. d. sinensis* lasted for 15 days. The eggs became lighter in color and translucent just before hatching. Newly hatched larvae look like the miniature version of the adult (Fig. 3) with an average TL of 2.3±0.5 mm. Figure 4 showed the number of larvae

Fig. 2: Appendix masculina (arrow) on the second pleopod of matured male *N. d. sinensis*

Fig. 3: Newly hatched larvae of *N. d. sinensis*

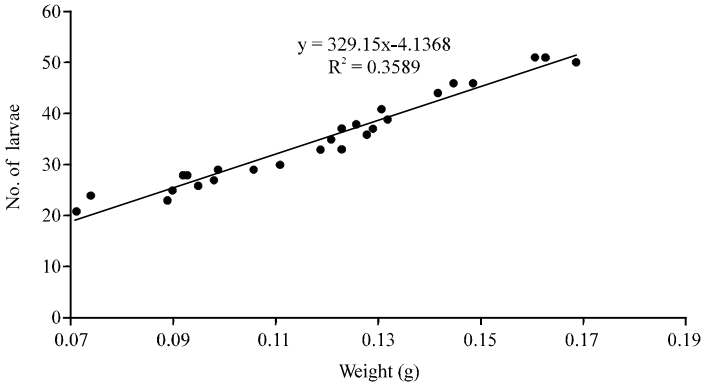

Fig. 4: Linear regression of fecundity (number of larvae produce) based on weight of female *N. d. sinensis*

produce by females. It ranged from 21-51 larvae per hatching, increased linearly ($R^2 = 0.9587$) with the size of the female. Larvae reached juvenile stage after 60 days. At this stage, male and female were undifferentiated. Juveniles take 15 days to reach adulthood and first maturity. Complete life cycle of *N. d. sinensis* is presented in Fig. 5.

During the study period, it was found that larvae and adult shrimp cultured in freshwater were susceptible to clitellate annelids, *Holtodrilus* sp. (Fig. 6) resulted in high mortality.

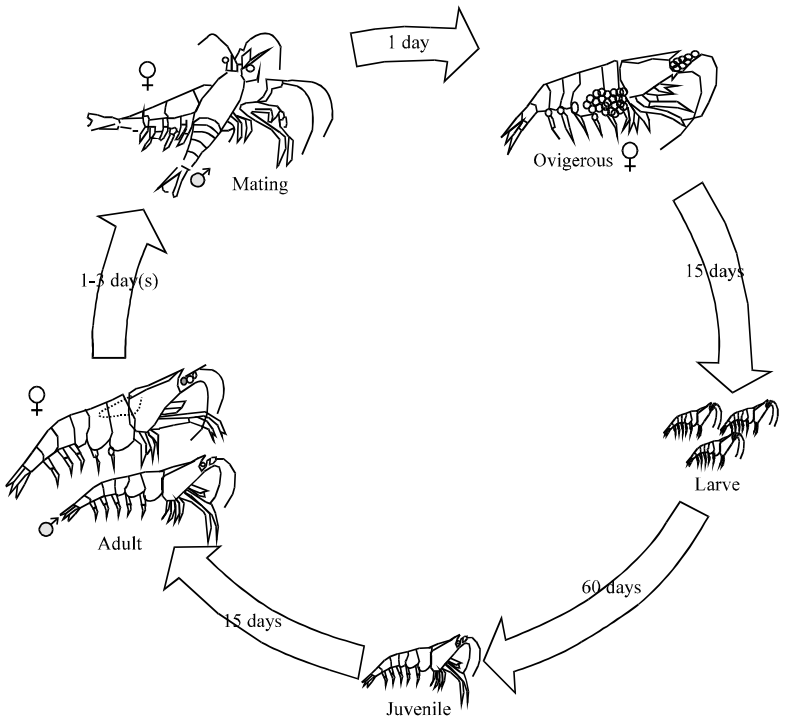

Fig. 5: Life cycle of *N. d. sinensis*

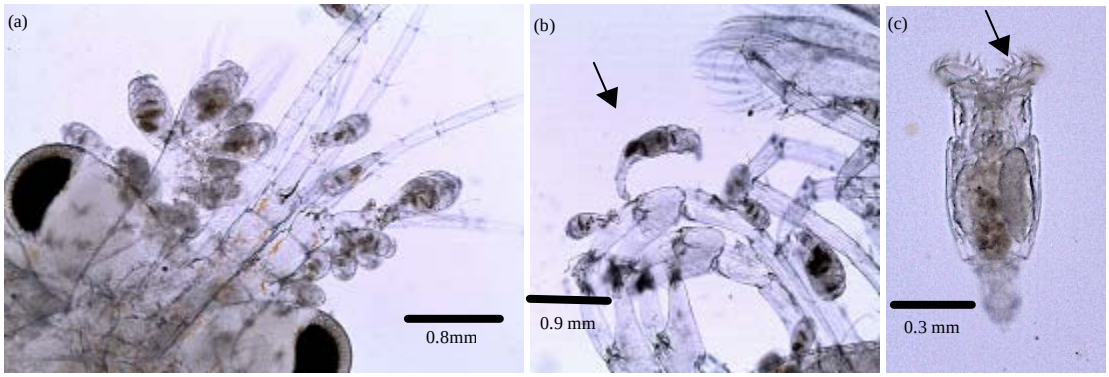

Fig. 6(a-c): *Holtodrilus* sp. were distributed all over the body surface of *N. d. sinensis* cultured in freshwater, (a) *Holtodrilus* sp. attached to the naupliar appendages of larvae, (b) *Holtodrilus* sp. elongated (arrow) ready to move and (c) Tooth (arrow) on the jaws of *Holtodrilus* sp.

Table 1: Ranges of water quality parameters measured during the study period

Parameters	Range
Dissolved oxygen (mg L^{-1})	>4.0
Temperature (°C)	27-28
pH	6.5-7.5
Ammonia (mg L^{-1})	<0.1
Nitrite (mg L^{-1})	<0.1

Holtodrilus sp. with body length 0.5-0.6 mm were found distributed all over the body surface of the host, with a higher concentration around the appendages. Culture of this species in slight saline condition between 5-10 ppt can effectively treat this annelid. Water quality parameters like dissolved oxygen (DO) was more than 4 mg L^{-1}, temperature 27-28°C, pH 6.5-7.5 and ammonia and nitrite less than 0.1 mg L^{-1}, respectively (Table 1).

DISCUSSION

In this study, *N. d. sinensis* was found to be able to reached first maturity as early as 75 days old, which is similar to those reported by Demas (2007). Female molted prior to mating, possibly induced by the released of certain chemical substance into the surrounding water. Somehow, before or during molting, the release of this substance allows the male to detect gravid female in the water column. This signaled the male that female is ready to spawn.

Newly hatched larvae of *N. d. sinensis* look like the miniature version of adult similar to that observed by Yang and Ko (2003). Morphological differences were only observed when *N. d. sinensis* reached maturity with the appearance of endopod and appendix masculine in males, which is similar to the report by Englund and Cai (1999). *N. d. sinensis* lacked of planktonic larval stage, therefore, it is a completely suppressed type based on the type categorized by Lai and Shy (2009). Numbers of egg produced per female of freshwater shrimp is depending on the size of the female itself. Smaller females produced fewer eggs compared to larger females (Ketse, 2006). In the present study, the size (weight) of female *N. d. sinensis* had a linear and positive relationship ($R^2 = 0.959$) with number of larvae produced. Similar observation was reported on *Caridina gracilirostris* (Heerbrandt and Lin, 2006).

Female *N. d. sinensis* was observed to incubate and fan their eggs in the brood pouch. According to Adiyodi and Adiyodi (1994) this kind of parental care will results in higher survival rate of larvae produced. Similar to those reported by Schram (1986), *N. d. sinensis* eggs were incubated in brood pouch until hatching.

Infestation by clitellate annelids on *N. d. sinensis* is quite common. Similar observation on others freshwater shrimp was reported by Liang (1963) and Liu (1984). This study suggested it is better to culture *N. d. sinensis* larvae in slightly saline water (5-10 ppt) since clitellate annelid, *Holtodrilus* sp. will not be able to tolerate saline water. Study by Niwa *et al.* (2005) stated that clitellate annelids, *Holtodrilus truncatus* infestation on freshwater shrimp can be treated with saline water.

CONCLUSION

Neocaridina denticulata sinensis can successfully breed in laboratory conditions using a simple experimental setup. This shrimp is able to reach first sexual maturity earliest at 75 days old. It reproduces throughout the year. It is recommended to culture newly hatched larvae in slight saline

water (5-10 ppt) to avoid infestation and mortality due to clitellate annelid. The findings of this study can be used as a guideline for culturist interested in the mass production of this ornamental shrimp for the aquarium industry.

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