Influence of adult male on sexual maturation of male and female larvae in the gnathiid isopod *Elaphognathia cornigera*

Katsuhiko Tanaka

**Abstract.**— Responses of larval individuals of the fish-parasitic gnathiid isopod *Elaphognathia cornigera* under presence or absence of adult male were examined by a laboratory rearing experiment. Female larvae molted into adults after 29.5 ± 4.8 days (mean ± s.d.) under male absence since the final parasitism against 17.4 ± 3.4 days under male presence. Ovulation in females was never observed under male absence during the experimental period while it occurred within a day after maturation of female larvae under male presence. The delayed molting and inhibition of ovulation under male absence are considered to provide additional time for finding a mate when the availability of males is low and to decrease reproductive failure of females. In contrast to females, solitary reared male larvae took less time to mature than those reared with a male. The delayed molting of male larvae under male presence was suggested to contribute to reduce potential attacks from adult males when male-male competition exists. Precopulatory mate guarding behavior by adult males was observed more frequently for female larvae than for male larvae. This indicates that adult males of the gnathiid can discriminate the sex of larvae as well as larvae recognize the presence of males.

**Key words:** availability of mates, delayed molting, precopulatory mate guarding, gnathiid isopod

**Introduction**

Mating opportunity in Crustacea is often restricted to a brief period after the female’s molt because females in many species are only receptive for a short time after molting until the exoskeleton has hardened, while copulations in others occur during the intermolt period when the exoskeleton is hard (Hartnoll, 1969). For example, the duration of female receptivity is limited to several hours after the molt for *Cancer gracilis* Dana, 1852 (Orensanz *et al.*, 1995), 5–12 hours for the portunid crab *Callinectes sapidus* Rathbun, 1896 (Gleeson, 1991), and about 1.5–3 hours for the caridean shrimp *Palaemonetes pugio* Holthuis, 1949 (Bauer & Abdalla, 2001). Isopod crustaceans molt the posterior part of the body before the anterior part, with copulation and insemination often taking place after the posterior molt (Wilson, 1991), and the duration of female receptivity was reported to be 6–50 hours after the female posterior molt for the marine isopod *Paracerceis sculpta* (Holmes, 1904) (Shuster, 1989).

The limited mating opportunity involves risks such as decreasing female reproductive success when females have difficulty to encounter mates during the appropriate period. In the gammarid amphipod *Gammarus palustris* Bousfield, 1969, the frequency of copulations decreased with time and the proportion of viable broods decreased even further if mating was delayed beyond 12–24 hours since the female’s molt, and the number of developing eggs per brood also decreased as time since the female’s molt increased (Borowsky, 1988). Fe-
males of the idoteid isopod _Idotea baltica_ Pallas, 1772 ovulate in the absence of a male, but delayed ovulation in those conditions, and such eggs disappear from the brood pouches a few days after oviposition (Borowsky, 1987). In iteroparous species, the failure of a single reproduction might be compensated by future fecundity and the loss of the lifetime reproductive success could be minimized. However, the low availability or absence of males would be critical in the female reproductive success in semelparous species.

Gnathiidae is an isopodan family with a biphasic life cycle that includes a fish-parasitic larval phase and a non-feeding adult phase, and females are known to be semelparous (Smit & Davies, 2004; Tanaka, 2007). Larval growth proceeds via several alternating periods of ectoparasitic feeding on host fish, followed by molting in benthic habitats, before individuals reach maturity. Larval gnathiids metamorphose into adults with a significantly different morphology (Smit & Davies, 2004; Tanaka, 2007). Adult gnathiids are regarded to reproduce in their benthic habitats without feeding, and females die after releasing larvae from their ventral brood pouches (Smit & Davies, 2004; Tanaka, 2007). Larval gnathiids move several times between hosts and the benthic habitat prior to the maturity, and passive movement depending on currents or host movement/migration may increase dispersal over large scales. Dispersal during the ectoparasitic phase of larvae is thought to affect the distribution pattern of benthic individuals, and larval gnathiids may be transported and settle in areas where conspecific individuals are scarce. However, responses of females in semelparous gnathiids under limited availability of males have not been studied.

The availability of adult males may also influence the future reproductive success of larval males when male-male competitions for females occur. In gnathiids, adults show strong sexual dimorphism (Smit & Davies, 2004; Tanaka, 2007), and males have large elongated mandibles often regarded to be weapons to monopolize and guard females (Upton, 1987; Klitgaard, 1997). Males of some gnathiid species form harems consisted of a male and several females (Upton, 1987; Wägele, 1988; Barthel & Brandt, 1995; Klitgaard, 1997; Tanaka & Nishi, 2011). Furthermore, a male-male struggle can cause the death of a male in the Antarctic gnathiid _Caecognathia calva_ (Vanhöffen, 1914) when two males are kept in a laboratory tank (Wägele, 1988). The presence of adult males may be unfavorable for larval male gnathiids just before maturity, and the behaviors of larval males under male presence/absence would be important to better understand the male mating strategies.

_Elaphognathia cornigera_ (Nunomura, 1992) is a gnathiid species inhabiting intertidal encrusting sponges. The complete life cycle shuttling between fish hosts and benthic habitats three times, thus, bearing three larval stages, was clarified, with the intermolt period of adult females being relatively short (12–20 days) in gnathiids, based on field and laboratory data (Tanaka & Aoki, 1998; Tanaka, 2003). A laboratory rearing technique has been established (Tanaka, 2003). Therefore, the species is suitable to examine the responses and behaviors under controlled conditions in the laboratory. In the present study, larvae of _E. cornigera_ were reared in the laboratory under conditions of male presence or absence. The duration between the final parasitism and the maturation molt in each sex of the larvae was recorded to assess effects of the adult male on maturation of the larvae, and the significance of the observed response of the larvae was discussed.

## Materials and Methods

Field sampling was carried out in an intertidal rocky shore of the innermost part of Oura Bay located on the southern end of Izu Peninsula, Japan (34°40’N, 138°57’E). In the shore,
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*Halichondria okadai* (Kadota, 1922), the most dominant sponge species within the shore, was collected on August 21, 2017. Sponges were stored in a cooler box, taken back to the laboratory and carefully dissected to sort out *Elaphognathia cornigera* inhabiting the sponges. In addition to sponges, the intertidal goby, *Chaenogobius annularis* Gill, 1859 known to be a natural host of *E. cornigera* (Tanaka, 2002, 2003), was also collected from the shore by using hand nets as the host of larval gnathiids reared in the laboratory.

Individuals of *E. cornigera* taken from sponges were classified into unfed larvae before the parasitism with a fully segmented thorax, post-feeding larvae after parasitism with a posterior thorax dilated with host fluid, adult males with forwardly protruding mandibles and females with ventral brood pouches containing eggs. Furthermore, second post-feeding larvae were separated from first and third post-feeding larvae based on their body size (see Tanaka & Aoki, 1998) and used for a laboratory experiment together with adult males.

Second post-feeding larvae were housed in individual petri dishes filled with filtered seawater and maintained until they molt into the unfed form of third (= final) larval stage. After the molt, unfed third larvae were placed in a plastic vessel (10 cm diameter and 12 cm depth) containing 200 mL of seawater along with an individual of *C. annularis* as in Tanaka (2003). Larvae left the host fish after feeding on the blood. Those post-feeding larvae with a dilated thorax were picked up and individually housed in a petri dish (45 mm diameter and 18 mm depth) solitary or together with an adult male until it metamorphosed into the adult. In each petri dish, a five-layered nylon mesh (mesh size: approximately 2 mm) fixed by a pair of plastic rings with 30 mm diameter was set as an artificial nest or substrate for the gnathiids as in Tanaka & Nishi (2011). All petri dishes and plastic vessels were maintained in an incubator at 25°C, similar to the sea surface temperature of the sampling site during the warm summer months, and water was daily exchanged.

During the daily exchange of water, the occurrence of the terminal molt from third post-feeding larva to adult was checked, debris on the body surfaces of gnathiids was removed by

*Fig. 1. Precopulatory mate guarding behavior of Elaphognathia cornigera.* A male clinging the dorsal side of a premature larva in the laboratory (a) and in the sponge habitat collected from the field (b). C: The outline of field specimens in B. M: adult male; L: premature larva.
using a fine nylon hair attached on the tip of a stick, and dead larvae were removed. When larvae metamorphosed into females, additional observations were made until 5 days later of the metamorphosis and the ovulation was daily checked. During the experiment, a male was observed to cling on the dorsal side of a larva in the artificial nest (Fig. 1a). This behavior was observed in sponge habitats collected from the field (Fig. 1b, c) and regarded to be a precopulatory mate guarding behavior of males. The guarding behavior was also recorded during the daily exchange of water.

Results

In the present study, total 36 individuals of third post-feeding larvae were examined. Of those larvae, 18 individuals were reared solitary and another 18 individuals were each maintained with a male. Among the solitary reared larvae, 9 and 4 individuals metamorphosed into males or females, respectively, and the other 5 died before reaching maturity (Table 1). When reared with a male, 7 larvae become males. Seven larvae did females and 4 larvae died during the experiment (Table 1). The sex ratio of successfully matured individuals was not significantly different between solitary larvae and larvae reared with a male (Fisher’s exact test, \( p = 0.19 \)).

For solitary reared male and female larvae, the duration from final parasitism to maturation molt was 8.9 ± 1.5 days (mean ± s.d.) and 29.5 ± 4.8 days, respectively, and the range was 7–10 days in males and 25–35 days in females (Table 1). When kept with a male, male larvae needed 12.0 ± 2.6 days to mature after the final parasitism (Table 1), and it was significantly longer than that of solitary reared male larvae (Mann–Whitney’s \( U \) test, \( N = 16, U = 11.5, p < 0.05 \)). Female larvae reared with a male took 17.4 ± 3.4 days until the metamorphosis after the final molt (Table 1). The duration was significantly shorter than that of solitary reared female larvae (\( U \) test, \( N = 11, U = 28, p < 0.01 \)). The duration to reach the maturity since final parasitism took a longer time in males and shorter time in females when they were reared with an adult male. Therefore, the difference of the duration among sexes become small when a male coexisted with larvae, but males tended to mature earlier than females after the final parasitism.

Precopulatory mate guarding behaviors by adult males were observed for 12 of 14 larvae which successfully metamorphosed into adults under male presence (Fig. 2). Five of the guarded larvae were males and others were females. The number of times that guarding behavior was observed was different among sexes of larvae, and was 1.9 ± 2.0 for male larvae and 6.6 ± 3.4 for female larvae. When standardized by the duration between the final parasitism and following molt, the frequency of the guarding behavior observed was 0.40 ± 0.23

<table>
<thead>
<tr>
<th>Larvae reared</th>
<th>Fate of larvae</th>
<th>N</th>
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<th>Days to mature or die since parasitism</th>
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<td>Mean</td>
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<td>Solitary</td>
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<td>50.0</td>
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<td>4</td>
<td>22.2</td>
<td>29.5</td>
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<td>Death</td>
<td>5</td>
<td>27.8</td>
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<td></td>
<td>Death</td>
<td>4</td>
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times per day in female larvae and 0.17 ± 0.20 in males, respectively, and significantly different among sexes (U test, N = 14, U = 41, p < 0.05).

All female larvae experienced the guarding behaviors of males during the experimental period, but the frequency was different among female larvae. The guarding behavior of males was observed only three times for two female larvae but over 10 times for three female larvae (Fig. 2). The former larvae took 17–18 days to mature against 17–22 days for the latter (Fig. 2). There was no significant relationship between the frequency of the guarding behaviors by males and days to mature in females after final parasitism (Spearman’s rank correlation coefficient, ρ = 0.009, p = 0.91).

Among individuals matured to adult females under male presence, four individuals had laid eggs in their brood pouches when their maturation were observed, and other three females ovulated by the next day after metamorphosis (Fig. 2). However, no ovulation occurred in females reared solitary during 5 days after metamorphosis (Fig. 2).

Discussion

During the present study, female larvae of *Elaphognathia cornigera* delayed molting when they were reared in a solitary condition. Such behavior has been reported for amphipod crustaceans (*Gammarus pulex* (Linnaeus, 1758), Ward, 1984, *Eogammarus oclairi* Bousfield, 1979, Iribarne *et al.*, 1995) and is often considered as a female adaptation for maximizing fertilization of eggs by providing additional time for finding a mate (Iribarne *et al.*, 1995). However, the time of delay in *E. cornigera* was approximately 12 days on average, which was longer than those reported as 3 days in *G. pulex* (Ward, 1984) and as 4 hours in *E. oclairi* (Iribarne *et al.*, 1995). In females of semelparous species like gnathiid isopods, a reproductive failure indicates that her lifetime reproductive success is zero, and the importance to increase chances to encounter males for the avoidance of the reproductive failure might reflect the longer duration of the delay of molting than in the previously studied iteroparous species.

Although oviposition of female *E. cornigera* occurred soon after the maturation molt of females under male presence, solitary reared females never extruded eggs into their ventral brood pouches in *E. cornigera* during the experimental period. The same pattern has been shown for several isopods and amphipods (iso-
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pods: *Asellus aquaticus* (Linnaeus, 1758), Thompson & Manning, 1981; amphipods: *G. pulex*, *G. locusta* (Linnaeus, 1758), Sexton, 1924; *G. fasciatus* Say, 1818, Clemens, 1950; *E. oclairi*, Iribarne et al., 1995). Suzuki et al. (2013) suggested that females of the intertidal isopod *Tylos granuliferus* Budde-Lund, 1885 must be newly inseminated just before each oviposition and receive some unknown substances inducing oviposition from males at the time of copulation. A similar mechanism might also exist in *E. cornigera* and affect the timing of oviposition. In some isopods and amphipods, females are able to lay eggs under male absence while the ovulation is sometimes delayed under the condition as in the isopod *Idotea baltica* (Borowsky, 1987). However, the viability of eggs often decreases as the time between oviposition and the following copulation increases (Borowsky, 1987). Oviposition under the male absence must involve a risk to decrease the survivability of eggs when females are not able to copulate immediately after the oviposition, and Thompson and Manning (1981) suggested that ovulation of females not guarded by males may be related to high availability of males. No oviposition after maturation molt in combination with the delayed molting under male absence in female *E. cornigera* may be a mechanism to maximize the fertilization of eggs when females have difficulty in encountering mates.

In a natural population of *E. cornigera*, males were observed throughout the year in the intertidal sponge *Halichondria okadai* (Tanaka & Aoki, 1998, 2000; Tanaka, 2003). They were abundant and the maximum density reaches nearly 40 individuals per gram sponge dry weight during the warm summer months when breeding females emerged (Tanaka & Aoki, 2000). The density of males often exceeds that of females during breeding periods (Tanaka & Aoki, 2000). Thus, the male limitation would rarely occur in the field population. However, intertidal zones are often exposed to strong waves or currents, and larval gnathiids swimming out to seek hosts may be washed away from the area where conspecific individuals are abundant. Furthermore, larval gnathiids attaching to fishes may be transported to areas where potential mates are scarce by movements of their host. Therefore, the parasitic behavior of gnathiid larvae may cause incidental dispersals which reduce the potential encounter of males and females. Responses of female *E. cornigera* are thought to avoid the decrease of reproductive success in such situation.

In contrast to females, male larvae of *E. cornigera* took significantly longer time to mature when they were reared with a male than when reared in a solitary condition. This might be related to the male–male competition in the gnathiid. Some species of gnathiids have been reported to form harems composed of a male and several females in their habitats (Upton, 1987; Wägele, 1988; Barthel & Brandt, 1995; Klitgaard, 1997; Tanaka & Nishi, 2011), and the forwardly protruding mandibles in males are often considered to be used to maintain and guard harems (Upton 1987; Klitgaard, 1997). Although little is known about male–male competition in gnathiids, Wägele (1988) observed that an intraspecific fight of the gnathiid *Caecognathia calva* led to the death of one of the rivals when two males were present in a laboratory tank. Therefore, delayed molting in larval males under the presence of an adult male might be an adaptation to avoid attacks by previously matured males as much as possible. However, in *E. cornigera*, harem formation has never been reported, and no direct observation on male–male competition has been made. Further investigations on male–male competition in *E. cornigera* will be needed to better understand the behavior of larval males under the presence of adult males.

Larval responses in gnathiids under male presence/absence would indicate the recognition of adult males. Additionally, the difference in the frequency of mate guarding behavior of
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males for male and female larvae indicates that adult males are also able to discriminate larval sex. The release of chemical signals to invoke increased mate searching or male-male contest has been described in crustaceans (see Dennenmoser & Thiel, 2015). In amphipods *G. pulex* and *G. duebeni* Lilljeborg, 1852, the urine of females contains a chemical substance that makes them attractive to males (Sutcliffe, 1992). Furthermore, larvae of the gnathiid *Paragnathia formica* (Hesse, 1864) tend to be attracted to mud containing adult males in the laboratory (Upton, 1987). The chemical signaling of larval and adult *E. cornigera* might contribute to recognize the presence of conspecific individuals and to discriminate the life stage and sex. However, there is no evidence of chemical communication in *E. cornigera*. This should be clarified in future studies.

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**Address**
(KT) Department of Marine Biology, School of Marine Science and Technology, Tokai University, 3–20–1 Orido, Shimizu, Shizuoka City, Shizuoka Prefecture, 424–8610 Japan

**E-mail address of corresponding author**
(KT) ktanaka@tokai-u.jp