Journal of Experimental Marine Biology and Ecology 486 (2017) 305–313

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Unraveling mating behavior for Axiidea (Crustacea: Decapoda): Burrow-dwelling callianassid shrimp in intertidal sandflat

Rei Somiya, Akio Tamaki *

Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Bunkyo-machi 1-14, Nagasaki 852-8521, Japan

ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 24 September 2016
Accepted 30 September 2016
Available online 2 November 2016

Keywords:
Axiidea
Burrow dweller
Callianassidae
Decapod crustacean
Intertidal sandflat
Mating behavior

ABSTRACT

Mating behaviors and mating systems in decapod crustaceans have attracted significant attentions. Dendrobranchiata and several infraorders of Pleocyemata (Caridea, Achelata, Astacidea, Anomura, and Brachyura) are the focal taxa. Virtually nothing is known about the members of Thalassinidea (recently separated into Axiidea including Callianassidae and Gebiidea including Upogebiidae) due to observational difficulties for their deep burrow-dwelling habit. Giving a little sediment and minute artificial tubes for one male and two females of the callianassid, *Nihonotrypaea harmandi*, in small transparent containers under illumination, observations and video-recordings of mating behaviors were made for the one pair three times, for the first time for Axiidea. The combined time schedule for each behavioral component was obtained. In inactive states, the shrimps stayed in their own burrows. The pre-mating visit was initiated by the male 3–4 d before the copulation, in which mutual signaling between sexes with movement of antennules, maxillipeds, chelipeds, and pleopods occurred. The final access was made by the hard-shelled female. The copulation lasted 91–105 s, with male onto female, during which a single spermatophore was transferred to sternite 8 surface with no sperm-storage structure. After the copulation, intimate exchanges occurred for 3–14 min. The female then isolated herself to an enclosed space for 60–74 min, during which oviposition started 44 min after the copulation, with embryo attachment to pleopods 1–2 completed in 12 min. The embryos were carried for 13–19 d before hatching. These findings would become basic to the understanding of thalassinidean shrimp population dynamics conducive to their key roles as benthic community organizers and ecosystem engineers in marine soft sediments.

1. Introduction

Mating behaviors and mating systems in decapod crustaceans have attracted significant attentions (Hartnoll, 1969; Salmon, 1983; Duffy and Thiel, 2007; Asakura, 2009; Bauer, 2011). The order Decapoda comprises two suborders, Dendrobranchiata and Pleocyemata. Of the latter, Caridea, Astacidea, Achelata, Anomura, and Brachyura are the focal infraorders. The members of two infraorders, Axiidea including Callianassidae (commonly ghost shrimp) and Gebiidea including Upogebiidae (mud shrimp), have completely been missed in the study of mating except for one brief description on copulatory behavior of a mud shrimp in the laboratory (Candisani et al., 2001). Ghost and mud shrimps are well known for their pronounced key roles as ecosystem engineers, community organizers, and pests for aquaculture operation in marine sedimentary habitats (Felder, 2001; Atkinson and Taylor, 2005; Pillay and Branch, 2011). Although Axiidea and Gebiidea have been lumped as Thalassinidea for a long time, recent molecular phylogenetic analysis has separated it into those clades (Robles et al., 2009; Dworschak et al., 2012). The former view of a single monophyletic infraorder was based largely on convergent adaptations to independently derived fossorial lifestyles in sand, mud, gravel, and coral rubble (Dworschak et al., 2012). The primary cause for the lack of observations on mating behaviors for ghost and mud shrimps is that fossorial lifestyle within their generally deep burrows. Individuals of most species live solitarily in their burrows (Dworschak et al., 2012) except for those of a few pair-bonding species (MacGinitie and MacGinitie, 1968; Berrill, 1975; Dworschak and Ott, 1993; Shimoda et al., 2005; Kneer et al., 2008). Laboratory observations may have been done using transparent aquaria with sediment, but all attempts ought to have resulted in failure.

In the present study, giving a little sediment and minute artificial tubes as burrow material for one male and two females of the callianassid, *Nihonotrypaea harmandi* (Bouvier, 1901), in small transparent containers under illumination, observations and video-recordings were made successfully on a series of pre-copulatory, copulatory, and post-copulatory behaviors by one particular pair three times, with the second record most detailed. The latter behavior included oviposition, embryo incubation, and larval hatching in the female. In light of convergence of mating behaviors and systems in Decapoda (Asakura, 2009), any characteristics about *N. harmandi* were extracted from the behavioral components and

* Corresponding author.
E-mail address: tamaki@nagasaki-u.ac.jp (A. Tamaki).

http://dx.doi.org/10.1016/j.jembe.2016.09.019
0022-0981/

associated systems that were found for species of some other infraorders of Pleocyemata. Morphological characters and life-history traits that might be linked with components of those behaviors were also noted.

2. Materials and methods

Individuals of *Nihonotrypaea harmandi* inhabit intertidal sandflats, residing solitarily in a Y-shaped burrow reaching up to 60 cm below the sediment surface; note that the name *Callianassa japonica* was incorrectly applied to *N. harmandi* in former papers (see Manning and Tamaki, 1998). Each burrow is composed of two surface openings, the swelling node of the Y situated at a mean depth of 10 cm (for adults), and several turnarounds (space for turning) at intervals below that node (Tamaki and Ueno, 1998). The shrimp feeds on phytoplankton and benthic microalgae contained in sediment that drops through the surface burrow openings (Shimoda et al., 2007). The shrimp matures at 20-mm total length (TL: curvilinear mid-dorsal length from rostrum to telson tips) 1 yr after larval settlement, with the beginnings of major-cheliped accelerated growth in male and of ovigerous female occurrence (Tamaki et al., 1997; Shimoda et al., 2005; Kubo et al., 2006). Both sexes have an indeterminate growth pattern up to the 2-yr life span (Tamaki et al., 1997). In female, a pair of close longitudinal ovarian ducts run along the mid-dorsal line from mid-cephalothorax posteriorly. In their most extended state, red-colored ova occupied the ducts to mid-pleomere 6, which is clearly visible through the translucent dorsal cuticle. The gonopores are located at coxa of pereiopod 3 in female and of pereiopod 5 in male. Embryos are attached to pleopods 1 and 2. The mean number of embryos per female is 333 (Tamaki et al., 1997). It takes 13 to 22 d for the embryos to develop to the time of hatch depending on water temperature (Tamaki et al., 1996). Consecutive broodings can occur, following larval hatching and the subsequent molting by females with well-developed ovary (Tamaki et al., 1996). Seasonally, ovigerous females occur from early June through October (Tamaki et al., 1997). In male, pleopod 1 is a simple two-articulated bud, and pleopod 2 is absent.

Adults of *N. harmandi* were collected from an intertidal sandflat in Koyagi, Nagasaki (129°47.4′E, 32°41.4′N) on 9 April 2015. One male (Male) and two females (Females A, B) were used for the laboratory observation spanning 146 d from 9 April to 1 September 2015. Their TLs were 34.7 mm (Male), 25.4 mm (Female A), and 34.8 mm (Female B). Either one or both of the females were reared with Male in a container in varying time segments, and in the former case, the other female was isolated to another container (Table 1).

Transparent polystyrene cylindrical cups (diameter × height in mm: 80 × 40 or 100 × 65) were used as containers. The cups were placed on a large transparent acrylic box that can accommodate one person. Field-collected sediment, with grain-size composition of 2.34 in median phi and 0.48 in arithmetic quartile deviation (well-sorted fine sand), was laid in 8–10 mm thickness on each cup bottom. Field-collected seawater was filled to a height of 30 or 50 mm. In most cases, one transparent polypropylene tube (termed tube: 14-mm diameter × 55-mm length) and/or two bottomless glass vials (10- and 19-mm bottom diameters × 45-mm height) were placed horizontally on the sediment for shrimps to utilize as their surrogate burrows. The seawater salinity was monitored with a refractometer (MASTER-S/Millα, ATAGO, Co.) and adjusted to 30–35 with tap water. The laboratory room was under natural temperatures until 8 July; the values in the cup water were monitored with a digital thermometer (SK-1260, SATO, KEIRYOKI MFG., Co.) once a day at irregular date intervals from 13 June to 8 July (Table 1). After 8 July, when the value reached 25.0 °C, the room was air-conditioned so that the water temperature was within 20.0–24.6 °C for maintaining shrimp normal states; the values were recorded once between 8:00–12:00 (mostly at around 10:00) daily as a rule. Foods were put onto the sediment, including pieces of green algae (*Ulva pertusa*) and small quantities of concentrated diatoms (*Chaetoceros gracilis*) and dead *Artemia* nauplii. Until 8 June, illumination was controlled daily by on/off of the fluorescent tubes on the ceiling every 12 h, with 'on' during 08:00–20:00 and 'off' during the rest. Thereafter, the room was continuously lit until the final date. Measurement for the reproduced setup (with Compact-LW, JFE Advantech, Co.) recorded 7–20 μmol quanta m^{-2} s^{-1} close to the container in the 'on'-phase. When the observation of shrimp behaviors was made from below the container bottom, it was also lit with a small fluorescent lamp from there, with 17 μmol quanta m^{-2} s^{-1} around that bottom (reproduced setup value).

Until 4 June, when the first brooding by Female A was noticed, shrimp behaviors were observed for varying durations at any time after 08:00 within each daytime morning at irregular date intervals. Thereafter, observations were made almost everyday and extended to the other times of each date when necessary. On selected occasions, fixed or handheld motion-digital video-recordings for varying durations were made for distinct events, including molting, wandering, mating (pre- to post-copulatory behaviors), oviposition, and larval hatching, by using a maximum of three cameras (HDR-CX500V, Sony, Inc.) sometimes with stereomicroscope objective lenses (DF plan 1× or 2 × $_{-2}$, Olympus, Inc.) attached for zoom (Table 1). These cameras were positioned above (V_1), aside (V_2), and below (V_3) the container. Pictures taken simultaneously from the different directions were edited with Vegas Pro 13 (Sony, Inc.) and representative captured shots shown in the figures.

3. Results

3.1. General burrow structure, visibility of behaviors, and food conditions

The natural burrow made by shrimps and the artificial tube burrow served as their hiding sites for substantial durations. The vials were used only transiently as a passage or temporal shelter. Natural burrows were frequently reconstructed, in which the least amount of sediment obliged the shrimps to make a short simple horizontal structure. A typical natural burrow had 1 swelling part (turnaround) inside and 1–4 open ends that were closed and reopened. The burrow wall was composed of thin sediment layers. In cross section, the burrow void space was circular or dome-shaped, with its diameter or height tailored to the shrimp's pleon height plus pleopod length. Shrimps in their burrows were visible at around open ends from above and aside (V_1 and V_2). In some cases, un-walled portions occurred on the burrow bottom, through which shrimps were visible from below (V_3). The shrimps appeared indifferent to the illumination. The shrimps in inactive states usually stayed within their burrows. When becoming competent toward the mating, they also moved around outside. Male and Female B appeared there more often than Female A. Except for her brief visits to other burrows, Female A stayed in her burrow. The adequacy of food conditions for shrimp gonadal growth was unknown, but at least Female A had three bouts of new broods over time. Female B had no broods despite maintaining well-developed ovary.

3.2. First brooding and hatching of larvae in Female A

All shrimps were placed in a cup on 9 April. Male and Female A made their natural burrows, and Female B occupied the tube. Male, Female A, and Female B molted first on 11 June, 16 May, and 26 April, respectively (Table 1). Including observations on other occasions, the componential time intervals in one molting sequence were approximately 5–10 min for ecdysis per se, 20–25 min for change from powerless (seemingly soft in exoskeleton) to normal active state (hard), and 53–84 min for discard of an exuvia out of the burrow. All shrimps hid in their burrows during 26–29 May. No observations were made on 30 and 31 May. Male was found to move around outside on 1 June, when Female A had no brood. No observations were made on 2–3 June. The presence of