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

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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.

Key words: Axiidea; Burrow dweller; Callianassidae; Decapod crustacean; Intertidal sandflat; Mating behavior

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 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 $2\times_{\text{-}2}$, 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.

158 **3. Results**

159 *3.1. General burrow structure, visibility of behaviors, and food conditions*

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

176

177 *3.2. First brooding and hatching of larvae in Female A*

178 All shrimps were placed in a cup on 9 April. Male and Female A made their natural
179 burrows, and Female B occupied the tube. Male, Female A, and Female B molted first on 11
180 June, 16 May, and 26 April, respectively (Table 1). Including observations on other occasions,
181 the componential time intervals in one molting sequence were approximately 5–10 min for
182 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 embryos in Female A was noticed first on 4 June. The start of mating in early June accords well with that in the field (Tamaki et al., 1997). During 4–17 June, Male and Female B were isolated to another cup, for which video-recording was made on 11 June for 1 d. No mating behaviors occurred.

The video-recording for Female A was made during 15 June, 16:20–16 June, 07:40. At 01:21 on 16 June, the larval hatching took place. Applying the shortest embryo-brooding period of 13–15 d (Tamaki et al., 1996), the date of oviposition is estimated to have been 1–3 June. About 30 min before the larval hatching, she began rushing movements within the burrow (intermittent to-and-fro and somersault). The total number of these movement elements every 10 min was 2–5 in the preceding 120 to 30 min, after which it increased to 9, 12, and 13, respectively. At 30 s before the larval hatching, she was stabilizing herself in the burrow turnaround by pressing pereiopods 5 onto the bottom wall. The larvae were ejected backward to the outside in 31 s through one open end of the burrow by strong currents generated by pleopods 3–5. About 3 min after the larval release, Female A began to groom pleopods 1 and 2 with pereiopods 5. The grooming continued for 57 min, during which time embryo shells were removed and discarded out of the burrow by currents with pleopods.

3.3. Second brooding in Female A and associated mating behaviors

On 18 June, Male with his tube was transferred to the cup containing Female A (Table 1). This pair was maintained until 1 July. Female A molted and evacuated the exuvia from her burrow at 11:28 on 28 June. The video-recording was made during 28 June, 11:28–29 June, 00:29. The width of her ovary was one-fourth that in its full-grown state. Male touched

that exuvia first at 11:31, handling it intermittently for the subsequent 40 min. After that, he seemed to lose interest in it. Until 12:17, he repeatedly moved around outside and returned home. Meanwhile, he visited one open end of Female A burrow, put minor cheliped in her burrow, touched her, quickly beat pleopods 3–5 in a coordinated manner at a rate of ca. 0.3 s per stroke, with telson and uropods bent downward; when irrigating the burrow interior, these pleopods moved more slowly at rates of ca. 0.6–1.7 s per stroke. Afterwards, Male and Female A were mostly in their tube and natural burrows, respectively.

Nothing peculiar happened in both sexes until 2 July. From 2 to 14 July, Male was back in the cup with Female B, where, Male, after his second molt made on 3 July, was attacked by her, and his major cheliped propodus tip was lost on 10 July. From 15 July to 7 August, the three shrimps were kept in the cup that had contained Female A, with Male occupying the tube burrow. Meanwhile, (1) both Male and Female B molted on 4 August, with loss of both chelipeds in the latter, (2) Female B died on 7 August (with no attacks from the other shrimps), and (3) Male and Female A copulated on 26 July. The shrimp behaviors in the event (3) are detailed below, dating back to 15 July.

During 15–21 July, Male and Female A stayed in their tube and natural burrows, respectively, except for 15 July, when Male was moving around outside. In the mornings of 22 and 23 July, Male was moving around outside. The video-recording was made during 23 July, 16:40–24 July, 05:48. At the start, Male was staying by Female A burrow, whereas Female B was in the tube. Until 19:44 on 23 July, Male often put antennules in one open end of Female A burrow. He also often protruded major cheliped toward that open end, with at least one touch on the major cheliped of Female A confirmed. Male often beat pleopods 3–5 quickly. At 19:20, Female B left the tube burrow, which was re-occupied by Male. Male stayed there until the end, and Female B was outside. The above-mentioned open end of Female A burrow was closed by herself from inside at 0:07 on 24 July. It remained closed

until the end.

In the mornings of 24 and 25 July, Male and Female A were in their respective burrows. Around 17:00 on 25 July, only Male was outside. The video-recording was made during 25 July, 17:08–22:17 and 25 July, 22:23–26 July, 10:08 with V_1, 25 July, 17:44–26 July, 10:03 with V_2, and 25 July, 17:29 –22:14 with handheld V_3 and 25 July, 22:25–26 July, 09:56 with fixed V_3. Male entered Female A burrow from one open end at 18:54 on 25 July, when she rushed out of the burrow but soon returned within this minute. This encroachment by Male on Female A burrow resulted in partially destroying it, with the subsequent repair work done by himself and partly by her. They cohabited until 21:41, when Female B touched Male telson from the outside, and he rushed out of the burrow but soon returned to his tube burrow. At 00:01 on 26 July, Male, in the outside, closed another open end of Female A burrow. The closure continued until 00:24, when Female A opened that closed point. This reopened 'open end' and one open end of Male tube burrow were positioned close-by (Fig. 1A). Male visited that open end of Female A burrow twice, protruding his major cheliped toward it for 48 s in total, and returned home. Female A visited that open end of Male burrow twice for 2 min 55 s in total, during which she touched on his major cheliped with her wiggling maxillipeds.

At 00:37 on 26 July, Male and Female A finally proceeded for the copulation. Initially, she visited his tube burrow, faced him, touched on his major cheliped (carpus to propodus part) with her wiggling maxillipeds (Fig. 2A,B), turned her back on him, and stopped (Fig. 2C,D). He approached her from behind (Fig. 2E–H), overturned her with his pereiopods 2 and 3 (Fig. 2I–K), and copulated with her for 105 s (from 00:37:43 to 00:39:28), embracing her major cheliped by flexing his major cheliped at its carpus junctions with propodus and merus, and overlapping their sternites between pereiopods 3 and 5 (Fig. 2L). During the copulation, she was motionless except for pereiopod 5 (only one side was clearly seen), extending major cheliped anteriorly (cf. Fig. 2I–L). Her pereiopod 5 touched the base of the

outer surface of his pereiopod 4 150 times for that 105 s (Fig. 2L). Immediately after the copulation, he retreated back to his tube burrow, and she followed him to enter his burrow partially until 00:40:41 (as in Fig. 1C) and entirely until 00:41:54. Their behaviors inside were invisible. She then returned to her own burrow. While spermatophores were absent on her cephalothoracic sternum before copulation (at 18:56 on 25 July; Fig. 1B), a single stalked spermatophore (precise stalk length unspecified) with at least four distal lobes or ampullae (precise number unspecified) was found attached to a central sternite 8 immediately after copulation (at 00:39:46 on 26 July; Fig. 1C) and before oviposition (at 01:19 on 26 July; Fig. 1D). No thelycum-like structure for spermatophore attachment was discernible. See also Supplementary material: video, 'copulation', during 00:37:14–00:39:43 on 26 July, 2015.

The oviposition details in Female A are given below (see also Supplementary material: video, 'oviposition', at 8× speed during 01:22:16–01:47:49 on 26 July, 2015). At 00:42 on 26 July, Female A was bustling about in her burrow. At 00:58, as she transferred a portion of bottom sediment to the lateral burrow wall, the burrow inside became more visible from below (V_3). She moved about, shaking major cheliped vertically at short intervals and grooming pleopods 1 and 2 with pereiopods 5. At 01:18, the ova had moved from pleomeres 5–6 anteriorly. At 01:20:33, she closed the open end of her burrow that faced Male tube burrow. At 01:22:44, she began to bend the pleon ventral surface toward the cephalothorax. She shifted positions for the subsequent 27 s (Fig. 3A–E). At 01:23:13, she lay on her back and became motionless, stabilizing herself by pressing at least pereiopods 1 and 2 onto the wall of the enclosed space (Fig. 3F). At this time, the ova had further moved from pleomere 4 anteriorly. Then, ova gradually disappeared from the ovarian ducts, indicating the progress of oviposition (Fig. 3G–R). It ended at 01:25:25 (Fig. 3S). Pleopods 3–5 began to swing slowly at 01:35:29, when embryo deposition onto pleopods 1–2 was first confirmed owing to her having slightly changed postures. She remained still until 01:36:43. She then shifted

positions little by little (Fig. 3T–V). At 01:52, she began to repeat a longer shift with intermittent stops, with her bending posture maintained (Fig. 3W). At 02:03, she began to walk slowly, with her body stretched (Fig. 3X). At 02:21, she reopened her burrow at its foregoing closed point (Figs. 1A and 2). During the above event, Male stayed by Female A burrow for 9 min 7 s in total, and avoidance of or fight with Female B took place for 1 min 10 s in total.

3.4. Third brooding in Female A, and associated larval releasing and mating behaviors

From 27 July to the morning of 9 August, nothing peculiar happened in both Male and Female A with embryos derived from the second brooding. Her ova mass had regrown to reach pleomere 6 by the last date. Around 13:00 on 9 August, Female A was in her natural burrow, and Male was moving around outside. The video-recording was made during 9 August, 13:13–10 August, 03:40. At first, he was by her burrow open end that had been used at the time of the second brooding (Fig. 1A), with intermittent direct contacts with her, using his chelipeds. At 13:20 on 9 August, he was found inside her burrow. His encroachment on it should have been done through its another open end. From 18:44 afterwards, she was not seen in the burrow. He widened and extended the burrow, resulting in the position of the above-mentioned open end further closer to the open end of the tube burrow. In the morning of 10 August, she was in that tube burrow. The video-recording was made during 10 August, 19:03–11 August, 03:46 and 11 August, 22:32–12 August, 06:23. In each duration, several contacts between sexes occurred around the open ends of their burrows, with no cohabitation.

The video-recording resumed at the night of 13 August, and continued during 13 August, 21:28–14 August, 08:33. At 01:08 on 14 August, the flux of larvae released by Female A was observed at the open end of her tube burrow. It took 30 s for that flux to cease. The mean ± SD water temperature during the preceding brooding period for 19 d was 22.4 ± 1.1°C (n =

18; Table 1). During the larval release by Female A, Male stayed at that open end twice for 3 s and 42 s each. At 01:14, he contacted her through the open end of the latter burrow for 28 s. Subsequently until the time of copulation (next paragraph), such contacts occurred 5 times, each with 15 to 45-s duration, and those from her approaching the open end of his burrow occurred 3 times, each with 19 to 29-s duration.

The copulation took place during 02:21:55–02:23:26 on 14 August (for 91 s). Just before this, Female A entered Male burrow, faced him, and turned her back on him. He then approached her from behind and overturned her. Immediately after the copulation, she returned home. During 02:36:41–02:37:10, she appeared at the open end of his burrow, where he, in his burrow, touched on her major cheliped with his minor cheliped. At that time, the ova had been condensed up to pleomere 4 in Female A. She closed the open end of her tube burrow during 02:45:11–03:59:12. Meanwhile, he was by her burrow 10 times, each with 10 to 71-s duration and 6 min 11 s in total. When she reopened her burrow at 03:59, she had embryos on pleopods 1 and 2. Although, until 21:40 on 14 August, he and she were in the natural and tube burrows, respectively, the exchange of their positions was observed at 10:15 on 15 August. This situation was maintained until 17 August. The next day, their burrows connected partially, which continued until 1 September.

The last video-recording was made during 31 August, 23:12–1 September, 11:15. Female A released larvae in 31 s from 05:33:29 to 05:34:00 on 1 September. The mean ± SD water temperature during the preceding brooding period for 18 d was 23.1 ± 1.0°C (n = 15; Table 1). At the time of larval release, she positioned herself at the location where her former natural burrow had existed (Fig. 1A), whereas Male, in his tube burrow, stayed at its open end during 05:33:48–05:35:13. About 40 min before her larval release, she began rushing movements within the burrow. The total number of these movement elements every 10 min was 3 and 4 in the preceding 60 and 50 min, after which it increased to 9, 10, 8, and 10,

respectively. Two min 25 s after her larval release, she began to groom pleopods 1 and 2. At this time, her ovary was poorly developed. No copulation took place.

4. Discussion

Using one male and two females of *Nihonotrypaea harmandi* confined to a small container with a little sediment and minute artificial tubes under illumination, the present laboratory observation has for the first time detailed a series of pre-copulatory, copulatory, and post-copulatory behaviors for shrimp of the infraorder Axiidea. Even for shrimp of (former) Thalassinidea, only one brief description of copulatory behavior is available for *Upogebia noronhensis* (Gebiidea) in a sediment-filled aquarium (Candisani et al., 2001), as follows: “The process started with the male digging a straight and almost horizontal 10 cm long connection from the U part of its burrow to the U part of the burrow of the female. As soon as the connection was completed, the male and female immediately paired their ventral parts within the U-part of the burrow, both lying with the carapaces turned to opposite sides. The animals remained almost immobile for nearly 30 min, only gently moving the pleopods. After separation, the male moved back to its burrow and promptly started to close the connection.” With their vertical burrows embedded in sufficient volume of sediment, the communication of solitary ghost or mud shrimps with conspecifics is initiated by partially holing others’ burrows (Candisani et al., 2001; Shimoda et al., 2005). In the present experimental circumstances, such communications could conveniently be made via open ends of the horizontal burrows by shrimps (mainly the male) moving around in the opening between these burrows. While the light condition may influence shrimp normal behaviors, eyes are usually small or degenerate in Axiidea, and their function as light sensory organs is questioned (Dworschak et al., 2012). In fact, the present shrimps appeared to behave with no hesitation in that illuminated opening.

A series of pre- to post-copulatory behaviors performed by a pair of sexes constitutes a unique mating system for each decapod species in the presence of multiple conspecifics (Salmon, 1983; Thiel and Duffy, 2007; Asakura, 2009; Bauer, 2011). For shrimp of (former) Thalassinidea, no direct observations have been made on their mating system. By analyzing two microsatellite loci for embryos in females, Bilodeau et al. (2005) suggest polyandry in a population of the callianassid, *Callichirus islagrande*, with 20% of the specimens showing multiple paternity. Callianassid shrimp are known for their distinct sexual dimorphism in major cheliped, with male's weaponry (Dworschak et al., 2012). In *N. harmandi*, strong combatant behaviors using major cheliped were observed only between males in sediment-filled aquaria (Shimoda et al., 2005). Fights between multiple males would preclude normal mating behaviors in the present experimental setup. Even with the present only one male–two female setting, however, some suggestion could be obtained about the species mating system. In light of convergence of mating behaviors and systems in decapods (Asakura, 2009), it is worth searching for common behavioral components and associated systems among other infraorders of Pleocyemata. Any morphological characters and life-history traits that may be linked with components of the mating behavior are also noted.

The pre-mating visit was initiated by the *N. harmandi* male, starting 3.3–4.5 d before the time of copulation. The female receptivity through water-borne chemicals, if any, could have easily been detected by the male in the narrow container. In light of the process, mechanism, and adaptive significance of pre-mating chemical and (chemo)tactile communications between sexes in decapods (Atema and Steinbach, 2007; Bauer, 2011), the following phenomena are noted: (1) male's seemingly detecting act for female burrow interior, using antennules at open ends of that burrow, and the subsequent mutual touches between both sexes' chelipeds; (2) female's touch on male chelipeds with her maxillipeds; and (3) quick beating of male pleopods and maxilliped wiggling by the female possibly generating

383 'information currents' to send and receive mechanical and/or chemical signals through a
384 narrow burrow connection. The present male also exhibited some interest in the exuvia
385 ejected by the female. Male's interest in exuviae could be adaptive in locating a potentially
386 receptive female with full-grown ovary (Bauer, 2011), since such a female can be in phase
387 with larval release and the subsequent molt ready for the next brooding in this species
388 (Tamaki et al., 1996). The duration spent for pre-copulatory guarding of a female based on
389 male's assessment of that female's receptivity in the presence of other possible receptive
390 females and competing males is optimized in brachyuran crabs (Koga, 2007; Asakura, 2009)
391 and hermit crabs (Hazlett, 1968; Goshima et al., 1998; Asakura, 2009). It cannot be
392 determined whether the present male attendance with the female and the subsequent intimate
393 response exhibited by the female including a transient cohabitation can be regarded as the
394 male's pre-copulatory 'guarding' against (nonexistent) other males.

395 In the present case, the final access to the opposite sex toward the copulation was made
396 by the female. The behavioral sequence in the copulation from facing each other to the
397 male's overturning the female is the same as in two species of Astacidea (Farmer, 1974;
398 Atema et al., 1979). The duration for copulation per se was much shorter in *N. harmandi*
399 (91–105 s) than in the aforementioned *U. noronhensis* (30 min).

400 Female copulation in either hard (inter-molt) or soft (immediately after molt) exoskeletal
401 condition constitutes one reproductive strategy in decapods (Hartnoll, 1969; Raviv et al.,
402 2008; Asakura, 2009). The present *N. harmandi* female always did so in hard-shelled but
403 only when her ovary was full-grown either in non-ovigerous state or just after the larval
404 hatching. Based on a field experiment, Tamaki et al. (1996) suggest that soft-shelled *N.*
405 *harmandi* females could copulate. Those females with full-grown ovaries just after releasing
406 larvae and immediately undergoing the subsequent molt were enclosed with males in a small
407 container, which was buried in the sediment on an intertidal sandflat during daytime low tide.

One day later, a substantial proportion of females retrieved from multiple containers carried embryos on their pleopods, whereas those without males never became ovigerous. Since, in the present study, (1) the shrimp soft-shelled condition following ecdysis was estimated to last only about 30 min and (2) the copulation took place only at nighttime, it is uncertain whether truly soft-shelled females participated in the copulation in that field experiment. The present female did not molt immediately after larval release (first and third broodings).

Either external or internal spermatophore deposition on females constitutes another reproductive strategy in decapods (Hartnoll, 1969; Raviv et al., 2008; Asakura, 2009). This dichotomy is often accompanied by sperm storage either ephemerally for fertilization each time or over a protracted duration for multiple use (Sainte-Marie, 2007). It had been suggested for *N. harmandi* in the aforementioned field experiment that females must copulate at each egg fertilization (Tamaki et al., 1996). The present study clearly confirmed this as well as external spermatophore attachment. Inferring phylogenetic trends in decapod crustaceans based on male sperm transfer and female sperm storage structures was proposed (Bauer, 1986), in which the complete lack of information on (former) Thalassinidea is pointed out. It would not be very difficult to obtain spermatophore-bearing females (Fig. 1C,D), for which fine anatomy could be made.

The post-mating guarding of a female by a male would cost the latter less in decapod species with ephemeral sperm transfer externally on the former than in those species with each opposite trait (Koga, 2007; Asakura, 2009; Rasch and Bauer, 2016). Logically for the present *N. harmandi* male, the post-mating ‘guarding’ of the female from (nonexistent) other males would be needed for a maximum of 50–100 min up to her completion of embryo deposition onto pleopods. Rather than ‘guarded’ passively, the present female appeared to actively interact with the male for a while before starting oviposition. The female’s requirement for an isolated disturbance-free wide space during the oviposition was exhibited

433 by her closing the burrow and enlarging that space. The male never interrupted the female's
434 oviposition, merely staying by her closed burrow, with seemingly some concerns.
435 The aforementioned *C. islagrande* has thousands of embryos per female, and the
436 fertilized embryos derived from 2 or 3 males in the polyandrous females were separately
437 deposited onto anterior and posterior pleopods (to the 4th), in which those on the latter are
438 regarded as in suboptimal conditions (Bilodeau et al., 2005). In the present study, the time
439 available for a 'second' *N. harmandi* male to mate with the female is 22–44 min between
440 her copulation with the 'first' male and start of oviposition. If the mutual pre-copulatory
441 interactions between sexes for a substantial time is mandatory toward the successful
442 copulation, the deprivation of the spermatophore by that 'second' male and subsequent
443 interactions should be completed by the time when resorption of unused ova begins.
444 The present *N. harmandi* male consistently chose one of the two females, suggesting
445 monogamy in the mating system. This species promiscuity under natural conditions, if any,
446 does not occur among 'unlimited' number of mates as in free-ranging decapods (Koga, 2007;
447 Asakura, 2009; Bauer, 2011). In *N. harmandi*, due to spacing propensity between individuals
448 in high densities (Tamaki et al., 1997), the number of neighbors per shrimp is limited. For a
449 field population, the successive and synchronized occurrence of newly-ovigerous females
450 was recorded at a shortest interval of 2 wk, especially in phase with every spring tide after
451 mid-July (Tamaki et al., 1997). In such circumstances with receptive female numbers limited
452 at each spring tide, respective mate choice might not be strict for both sexes unless a great
453 size difference exists between them, resulting in the formation of each temporal pair as in a
454 hermit crab species (Goshima et al., 1998). One hypothesis about the *N. harmandi* mating
455 system is proposed here: serial monogamy in the potentially promiscuous situation among a
456 few inter-/intra-sexual members through the reproductive season.
457 Long-term pair bonding extended into the non-reproductive season is one mating system

458 in decapods, differentiated from temporal pair formation (Asakura, 2009; Bauer, 2011). In
459 non-reproductive seasons, heterosexual pairs co-occur in a same burrow in the callianassids,
460 *Nihonotrypaea petalura* in aquaria (Shimoda et al., 2005) and *Neotrypaea biffari* in the field
461 (MacGinitie and MacGinitie, 1968). Shrimp of both species dwell in burrows with
462 substantially wide interiors in low shrimp densities (< 10 m^{-2}) among the coarse sediment
463 of boulder shores (Shimoda and Tamaki, 2004; MacGinitie and MacGinitie, 1968). Each of
464 them constitutes a congeneric counterpart of tidal-flat inhabitants in high densities (hundreds
465 m^{-2}), *Ni. harmandi*/*japonica* and *Ne. californiensis*/*gigas*, respectively. For Axiidea, *Neaxius*
466 *vivesi* in gravelly sand (Berrill, 1975), *N. acanthus* in carbonate sand and coral rubble (Kneer
467 et al., 2008), and *Axiopsis serratifrons* in coral rubble (Dworschak and Ott, 1993) were
468 reported to form pair bonding, occurring in low densities (< 10 m^{-2}). Their burrows have
469 substantial wide space inside, accommodating 2 shrimps. The burrow openings are also wide.
470 Limited chances of finding mates should have been a major selective force for these five
471 pair-bonding species. The burrows in permeable sediments enable the residents to be
472 immersed in well-oxygenated water. Tidal-flat congeners subject to hypoxic pore waters in
473 finer sediments must have passages with a narrow diameter in their burrows (Felder, 2001;
474 Atkinson and Taylor, 2005), to which shrimp body heights are tailored for effective irrigation
475 currents with pleopods to flow smoothly (Stamhuis and Videler, 1998a, 1998b). Such narrow
476 diameters of their burrows would preclude cohabitation of 2 shrimps for a long time.
477 The present study has set the stage for future research on (former) thalassinidean shrimp
478 mating behaviors and mating systems that have been missed so long. The findings would
479 also become basic to the understanding of shrimp population dynamics conducive to their
480 key roles as benthic community organizers and ecosystem engineers in marine soft sediments.
481

482 **Acknowledgements**

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600 **Figure captions**

601 **Fig. 1.** *Nihonotrypaea harmandi*. **A.** Male and Female A in tube and natural burrows,
602 respectively, in the container, prior to their copulation in the second brooding; the time of
603 copulation was 00:37:43–00:39:28 on 26 July 2015. Their burrow openings were positioned
604 close-by. Video cameras V_2 and V_3 were set aside and below the container, respectively; V_1
605 (above container) is not shown. **B.** Female A with dorsal full-grown ovary taken with V_2
606 before copulation (at 18:56 on 25 July), with no spermatophore on sternite 8 (rounded in
607 white). **C.** Female A with a stalked and lobed spermatophore on sternite 8 (rounded in white)
608 taken with V_2 immediately after copulation (at 00:39:46 on 26 July). **D.** Female A
609 cephalothorax sternum, with a single spermatophore attached on central sternite 8 (arrow)
610 taken with V_3 before oviposition (at 01:19 on 26 July; original picture is rotated by 180°).
611 mxp: maxilliped. pp: pereiopod. gp: gonopore.

612

613 **Fig. 2.** Time series in the copulation process for Male and Female A of *Nihonotrypaea*
614 *harmandi* in their burrow setting (Fig. 1A) taken from aside (video camera V_2) during the
615 period from 00:37:14 (just prior to copulation) to 00:38:19 (in copulation) on 26 July 2015.
616 pp: pereiopod. See also Supplementary material: video, 'copulation'.

617

618 **Fig. 3.** Time series in the oviposition process for Female A of *Nihonotrypaea harmandi* in
619 an enclosed space of her burrow (Fig. 1A) taken from below (video camera V_3; container
620 bottom surface is seen) from before oviposition (01:22:47), through oviposition
621 (01:23:15–01:25:25), to embryo deposition onto pleopods 1–2 (01:35:29–01:36:43) on 26
622 July 2015 (original pictures are rotated by 180°). Each arrow indicates the direction from
623 telson to rostrum along dorsum, with bend postures in curves. Detached embryos/eggs are
624 indicated in circles (panels T–W). See also Supplementary material: video, 'oviposition'.

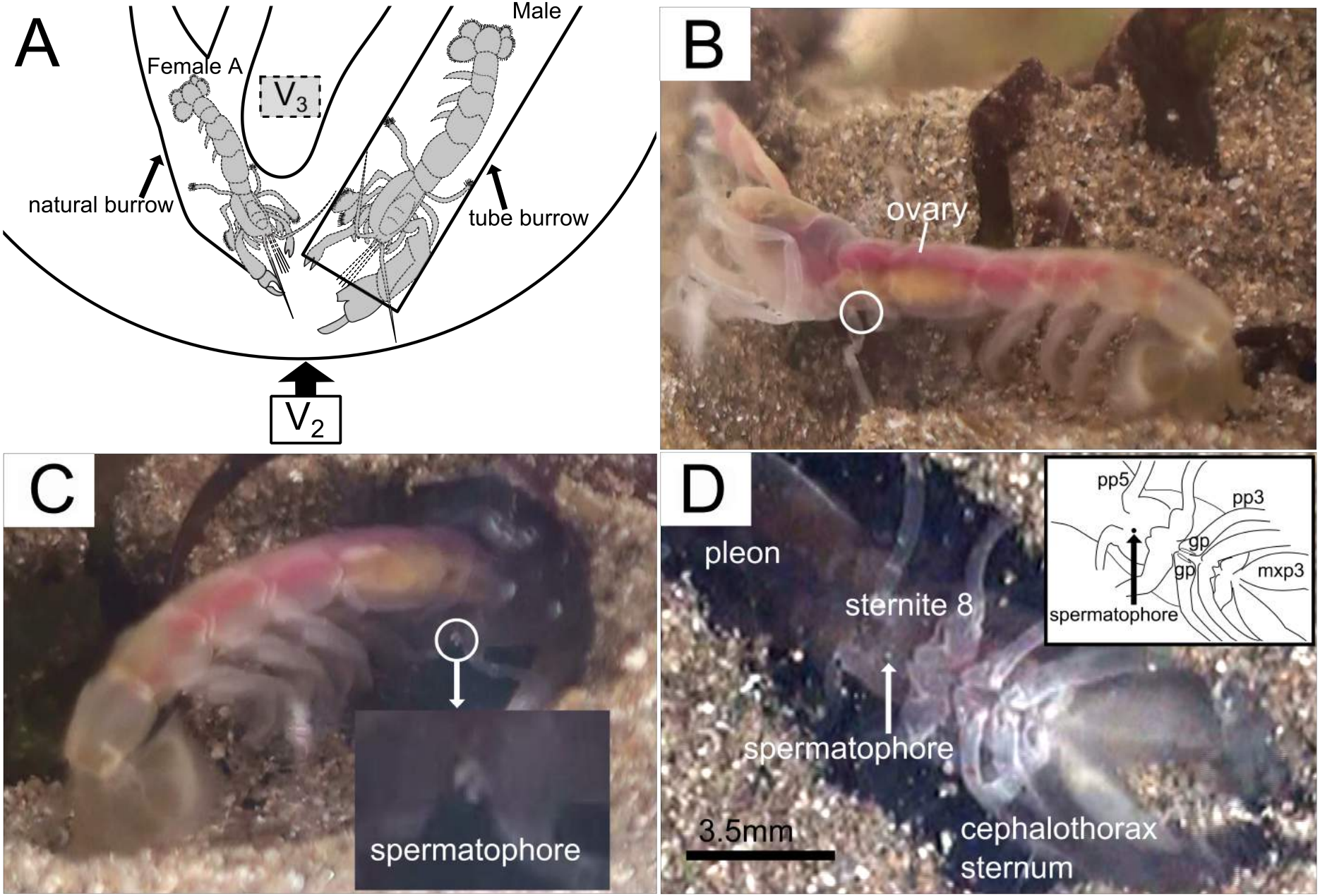

Fig. 1 (Somiya & Tamaki)

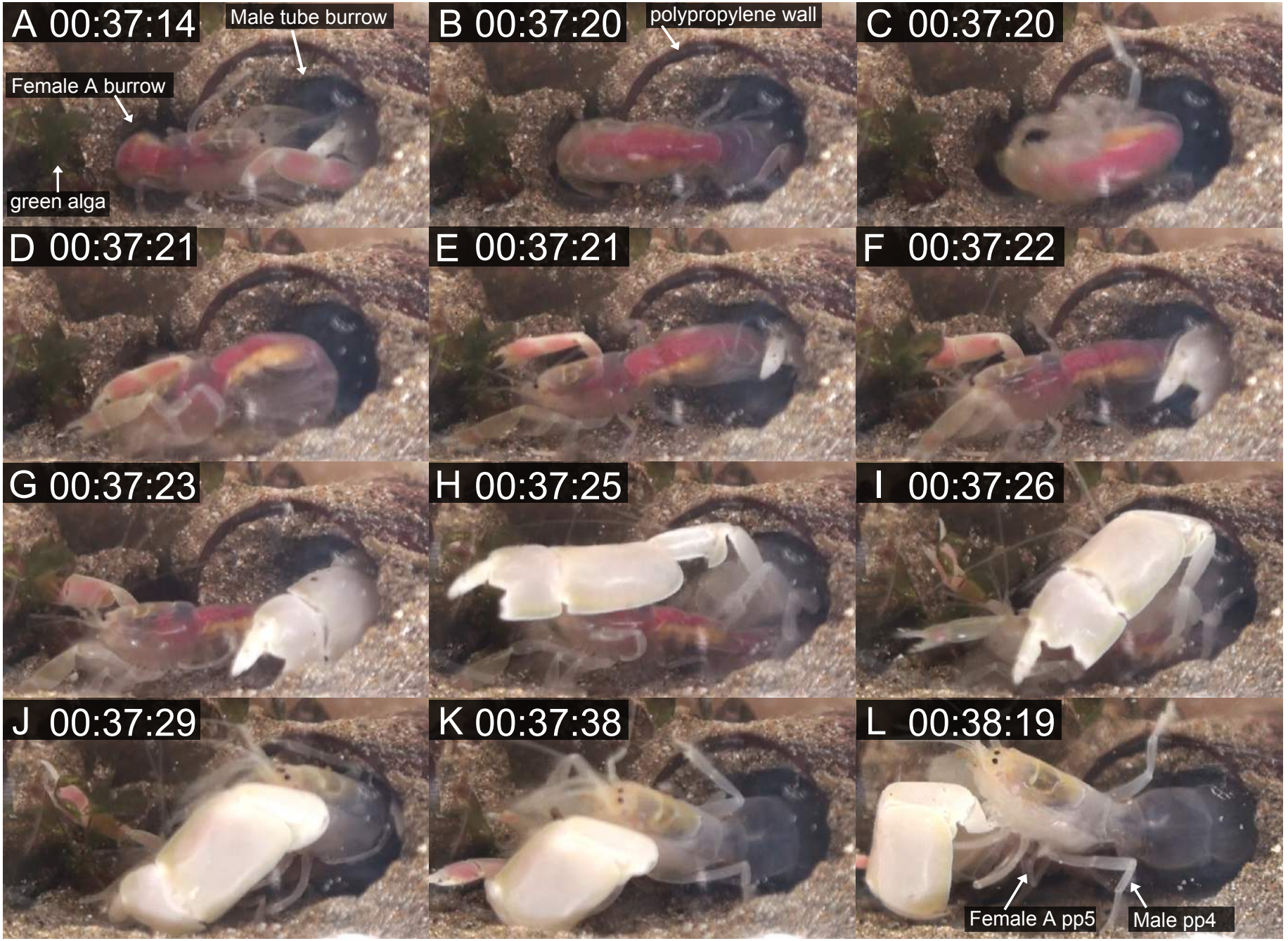

Fig. 2 (Somiya & Tamaki)

Fig. 3 (Somiya & Tamaki)

Table 1. Time series in events for Male and Females A and B of *Nihonotrypaea harmandi*, and in associated conditions and video-recordings in laboratory containers during 9 April to 31 August 2015. The embryo hatching (termed 'hatch') in the third brooding actually took place on 1 September (see text). The light was on/off every 12h from 9 April to 7 June, and thereafter continuously lit. From 9 July, the room was air-conditioned. The bars in water temperature indicate no data. The range with arrows and broken lines on 1–3 June indicates the unspecified dates for each event (see text). In Moon phase, open circle: full moon, solid circle: new moon, half-solid circle: half-moon.

Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Apr. (cumulative day no.)									1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	–
Moon phase				○								◑							●							◐					–
water temp. (℃)									–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Male (M)																															–
Female A (FA)																															–
Female B (FB)																										molt (FB)					–
combination									M+FA+FB																						–
May (cumulative day no.)	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Moon phase				○							◑							●								◐					
water temp. (℃)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
M																															
FA																molt (FA)															
FB																															
combination																															
June (cumulative day no.)	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	–
Moon phase			○							◑						●								◐							–
water temp. (℃)	–	–	–	–	–	–	–	–	–	–	–	–	24.0	23.5	23.7	23.5	23.5	22.8	–	23.5	23.0	–	23.5	–	24.1	–	–	–	–	23.8	–
M	<-----	mating	----->									molt (M)																			–
FA	<-	mating & brooding	->	brooding (1st)													hatch												molt (FA)			–
FB																		molt (FB)													–
combination					M+FB; FA														M+FA; FB												–
video-recording																															–
July (cumulative day no.)	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114
Moon phase		○							◑							●								◐							○
water temp. (℃)	–	24.0	–	22.8	22.9	–	24.0	25.0	22.5	23.8	22.7	–	24.6	21.4	24.2	23.1	22.4	20.0	20.8	20.1	22.3	22.8	23.1	21.5	21.4	22.5	21.5	23.9	22.8	23.7	–
M			molt (M)																							mating					
FA																										mating	brooding (2nd)				
FB																															
combination		M+FB; FA													M+FA+FB																
video-recording																															
Aug. (cumulative day no.)	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145
Moon phase							◑							●									◐							○	
water temp. (℃)	23.1	22.7	21.8	21.0	21.5	20.8	21.2	21.3	21.5	22.5	23.2	23.6	24.3	23.7	21.3	21.4	24.3	23.2	23.3	–	–	21.8	–	23.9	24.6	24.0	23.3	22.8	22.8	22.7	23.4
M				molt (M)										mating																	
FA	brooding (2nd)													hatch																	
FB														mating	brooding (3rd)																hatch
				molt (FB)			death (FB)																								
combination							M+FA																								
video-recording																															