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Reproductive phenology of an opilionid species maturing in early summer, *Himalphalangium spinulatum,* showing evidence of protogyny (Opiliones: Eupnoi: Phalangiidae)

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Abstract. The reproductive phenology of *Himalphalangium spinulatum* (Roewer, 1911) (Eupnoi: Phalangiidae), which overwinter as juveniles and mature in early summer, was surveyed at a riparian forest on the Sendai River, Tottori City, Honshu, Japan. Females became adults about 3–4 days earlier than males. The body weight of females and males and the number of mature eggs retained in females were highest at the age about a week after the final molting, suggesting a shorter time is needed to commence mating and oviposition in this species. This might be the reason for the shorter time lag in maturation between females and males in this species compared to other species of protogynous harvestmen.

Keywords: Sperm competition, life history, ovarian development
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One of the characteristic reproductive traits of harvestmen (Arachnida: Opiliones) is the prevalence of protogyny, which refers to the tendency for females to mature earlier than males in dioecious organisms (Ridley 1983; Thornhill & Alcock 1983). The occurrence of this trend in harvestmen was first reported by Tsurusaki (2003) for univoltine Eupnoi species that overwinter as eggs. Tsurusaki (2025) showed that females of *Odiellus aspersus* (Karsch, 1881) become adults about 10 days earlier than males. Tsurusaki (2003, 2025) attributed the cause of the protogynous tendency to the opilionid species having multiple mating and having a "cul-de-sac"-type spermatheca, which is essentially a diverticulum that has only a single gateway (Austad 1984), which may promote the last male's sperm priority in fertilization. However, no data have been available on whether protogyny is also exhibited in the species which overwinter as juveniles. It might be possible that harvestmen that overwinter as juveniles and mature in early summer do not show such a trend, because the long juvenile period spent over winter may alter the life history that would be expected in an egg-overwintering species.

To test whether a harvestman species that overwinter as juveniles also show protogyny, we surveyed the phenology of *Himalphalangium spinulatum* (Roewer, 1911), a univoltine species that overwinters as a juvenile and matures in late spring to early summer (Fig. 1, Tsurusaki 2015). This paper describes the results of the survey and presents an idea that the period needed for egg maturation in female adults might affect the degree of protogyny.

METHODS

The survey was made at a site in a riparian forest (Matsuoka & Sano 2003) on the right bank, called "Yasunaga-tei Bank," in a wide dry riverbed of the Sendai River (35.454838N, 134.213332E, Fig. 2). The forest consists mainly of Muku Tree *Aphananthe aspera* (Ulmaceae), Chinese Hackberry *Celtis sinensis* (Ulmaceae), and Japanese Timber Bamboo *Phyllostachys bambusoides* (Poaceae). The bank is said to be the remains of the bank constructed in the Edo Period (1600–1868) to control flooding of the Sendai River (Yoshimura 2006).

Himalphalangium spinulatum (Roewer, 1911) is a phalangiid species widely distributed in China, Korea, and western Japan (Suzuki 1950; Tsurusaki & Song 2000; Starega 2003; Tsurusaki 2015). Known localities of the species in Japan are scarce and have been only sporadically found in any part of Japan except for northern Kyushu. Unlike other harvestman species in Japan, this species does not occur in natural forests in the mountainous areas but tends to discontinuously occur in secondary forests, often found within bamboo forest surrounding villages (Tsurusaki 2015). This site in the riparian forest (Fig. 2) has been a single locality of the species known to date in Tottori Prefecture.

We (all the 4 authors together) looked for *H. spinulatum* for ca. 30 min. in the afternoon every week from May 2 to August 3, 2006 at the site (a rim of the riparian forest, Fig. 2B). Surveys after May 30 were intermittently made because we thought the data collected between May 9 and May 30 were already enough to examine the occurrence of protogyny in this species because no juveniles were found after May 23.

Specimens collected were immediately fixed and preserved in 80% ethanol. Using an electronic balance (minimum scale: 0.001 mg: LIBROR EB-3300; Shimadzu, Japan), we measured the body weight of each specimen fixed in 80% ethanol after removing extra ethanol completely from body surface with a filter paper. After the measurement of body weight, we returned the specimens to vials filled with 80% ethanol. We determined sex for juvenile specimens by dissecting the body and checking for a testis (male) or an ovary (female). Adults were sexed by checking presence (males) or absence (females) of longitudinal rows of ventromesal denticles on the palpal tarsus (cf. figs. 6–7 in Suzuki 1950).

After measuring weight, we dissected adult females in a petri dish filled with 80% ethanol in order to evaluate development of ovaries (cf. Fig. 3). Developmental stages were divided into four according to Tsurusaki (2003): Stage I (immature ovary) — Oocytes without any indication of yolk accumulation; Stage II — Yolks are accumulated in oocytes but mature eggs are not formed yet; Stage IIIa: — Mature eggs are retained in ovary, not in the uterus internus (i.e., egg reservoir); Stage IIIb; —Matured eggs can be found in both ovary and uterus internus (Fig. 3).

For statistical analyses, we used JMP, version 12.0.1 (SAS Institute 2015).

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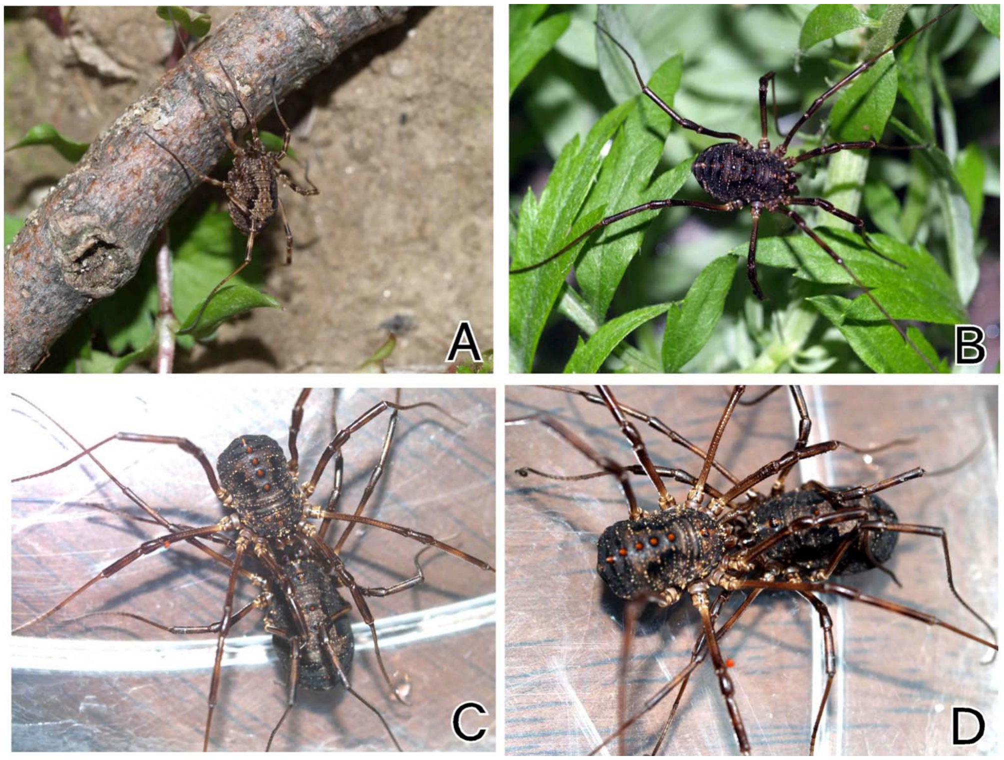

Figure 1.—*Himalphalangium spinulatum* (Roewer, 1911). (A) a juvenile. (B) a male, (C–D) a copulating pair (top in C and left in D is a male). Note that the male fixes the female's body by hooking his palps over at the base of the first pair of legs of the female. Hooking of the female's 1st leg bases is probably a prevailing feature in Phalangiidae (*Odiellus aspersus* also show this posture). Contrary to this, hooking female's base of the 2nd legs is typical in species of Sclerosomatidae.

RESULTS

We collected a total of 198 specimens of *H. spinulatum* in the surveys performed from May 2 to July 20 in 2006 (We concluded our series of the surveys because no specimens were found in the survey made on August 3). The numbers of individuals collected for juveniles (not sexed), adult males and adult females were as shown in Fig.4. The first *H. spinulatum* adults were found on May 23 for both males ($n = 2$) and females ($n = 8$) and the samples collected the following week (May 30) contained no juveniles. Unfortunately, only a single sample (23 May) contained both juveniles and adults. However, the difference in the adult-juvenile ratio between the sexes in the May 23 sample was significant (chi square test, <0.005).

Figure 5 shows the transitional curves from juvenile to adulthood estimated for females and males, separately, by checking the percentage of adults [Number of adults/(Number of juveniles sexed + Number of adults) $\times$ 100] for females and males, respectively. These curves suggest females mature 3 to 4 days earlier than males when compared at the point at which half of the individuals were adults, though data available were few because the survey was made only weekly and all the individuals of the species seemed to mature in a very short period, as shown in Fig. 6A. More than half of females collected on May 30, which

Figure 2.—Habitat of *Himalphalangium spinulatum* studied in Tottori City. (A) Kuniyasu-tei Bank (arrowed) in the right bank of the Sendai River. (B) The site where specimens of *H. spinulatum* were collected periodically in 2006.

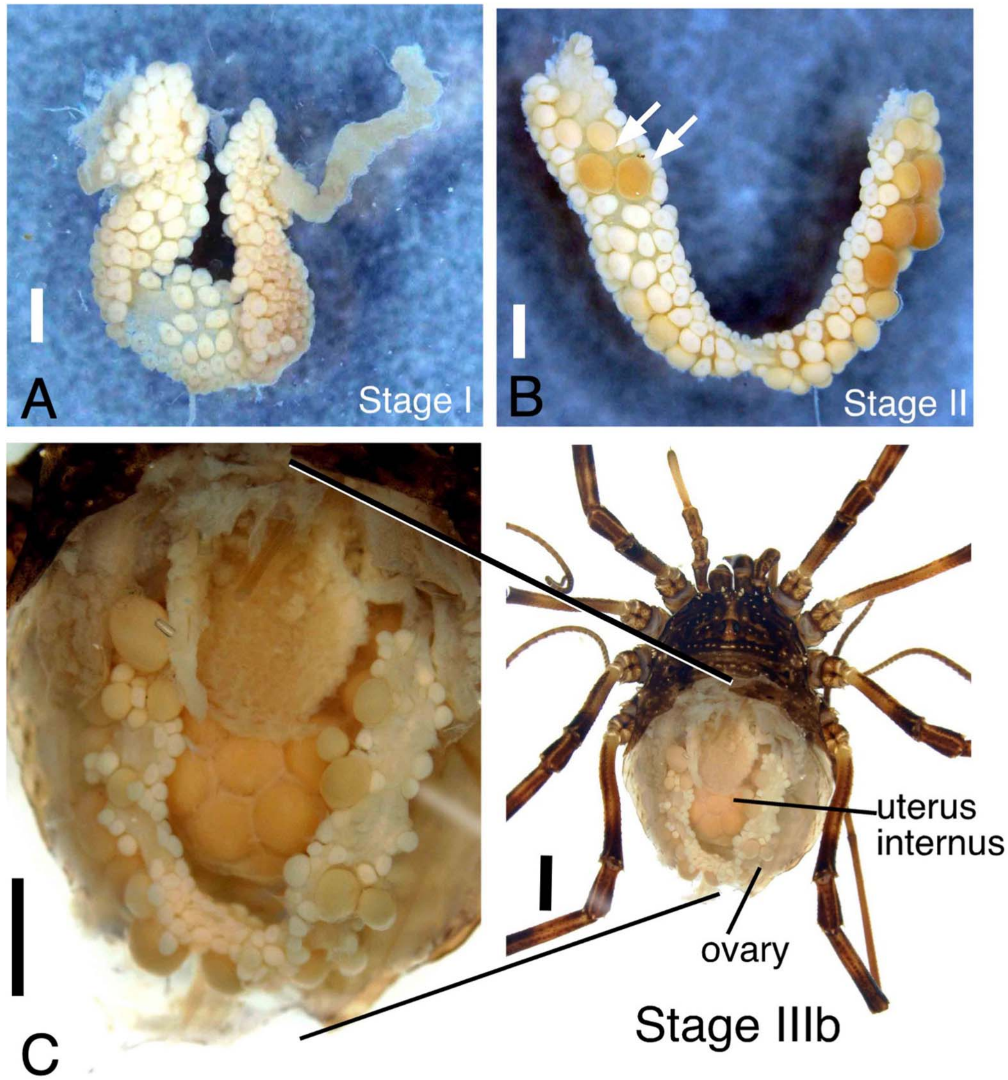

Figure 3.—Ovaries dissected out (A–B) and in situ (C) from adult females fixed in 80% ethanol. (A) Stage I: Ovary contains only immature eggs. (B) Stage II: Some eggs start to accumulate yolk (arrowed). (C) Stage IIIb: Uterus internus contains matured eggs. Scale bars = 2 mm.

is just a week later from May 23 when the first females appear, already had ovaries with matured eggs in the uterus internus (Fig. 6A).

The "uterus internus" (i.e., egg reservoirs, see figs. 4B and 7B in Tsurusaki 1982) is a sac where matured eggs, having been transferred from ovaries via the oviducts, are temporarily stored before oviposition. Having matured eggs in the uterus internus means that females are ready to oviposit. The number of eggs retained in the uterus internus was highest on May 30 (65 on average) and decreased after May 30, probably due to prior onset of oviposition (Fig. 6B). Body weight of males was also highest on May 30 probably due to development of testes or accumulation of fat stores related to the energy needed for searching females to copulate with.

As stated above, females retain a number of matured eggs in the uterus internus before oviposition and thus become much heavier as they mature. Body weight was measured (Fig. 7) under an assumption that body weight may roughly indicate the amount of investment to the next generation (accumulation of nutrients to eggs or sperm). Body weight measured for the specimens preserved in 80% ethanol may not be able to be used as such an indicator, because the content of 80% ethanol absorbed during preservation may not be consistent among different tissues. Fig. 8 shows the relationship of the number of eggs retained in the uterus internus to the body weight in females in the stage IIIb. There was a significant correlation ($r = 0.66$, $P < 0.005$) between body weight and the number of eggs. Thus, it seems that body weight measured for ethanol-fixed specimens can be used as a substitute for the body weight measured for living specimens.

DISCUSSION

Most of the species of egg-overwintering harvestmen of Eupnoi with a univoltine life cycle in Japan show protogyny (Tsurusaki 2003, 2025). This is remarkable because protogyny is extremely rare in insects, crustaceans, and spiders (Ridley 1983; Thornhill & Alcock 1983; Austad 1984). The prevalence of this trend in harvestmen is likely to be explained by the frequency of multiple

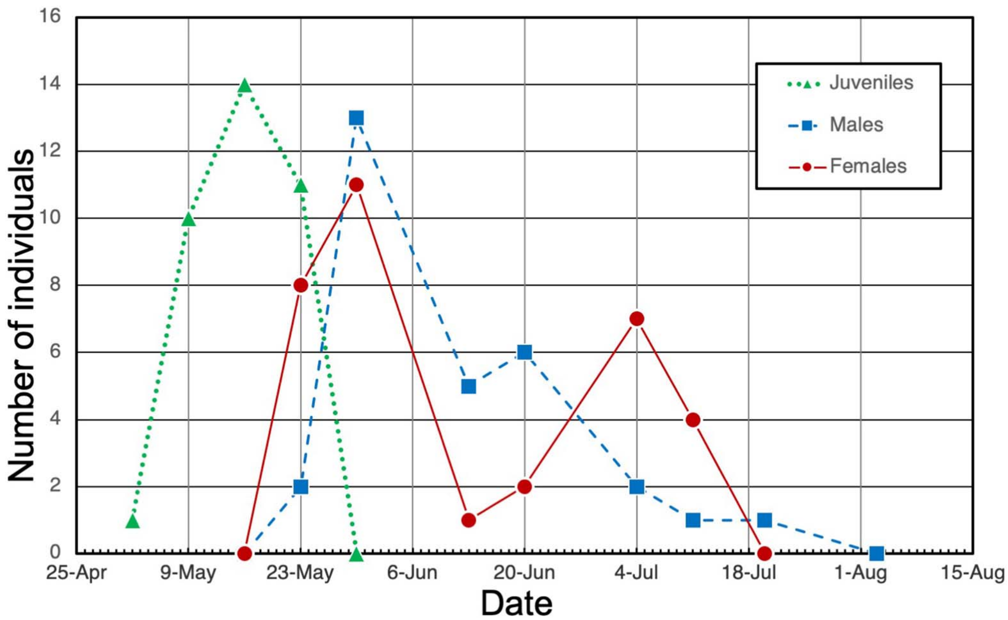

Figure 4.—The number of specimens collected as juveniles, adult males, and adult females. Difference in the cumulative numbers between males and females is significant (Kolmogorov-Smirnov 2 sample test, <0.001).

mating in both males and females (Machado & Macías-Ordóñez 2007; Fowler-Finn et al. 2018; Brown et al. 2020) and possible priority of the last mate's sperm in the fertilization of eggs in Eupnoi harvestmen (Tsurusaki 2003, 2025). However, no data on the difference between males and females in adult emergence have been available for the species that overwinter in stages other than eggs, except for a single adult-overwintering species, *Paraumbogrella pumilio* (Karsch, 1881) (Tsurusaki 2003).

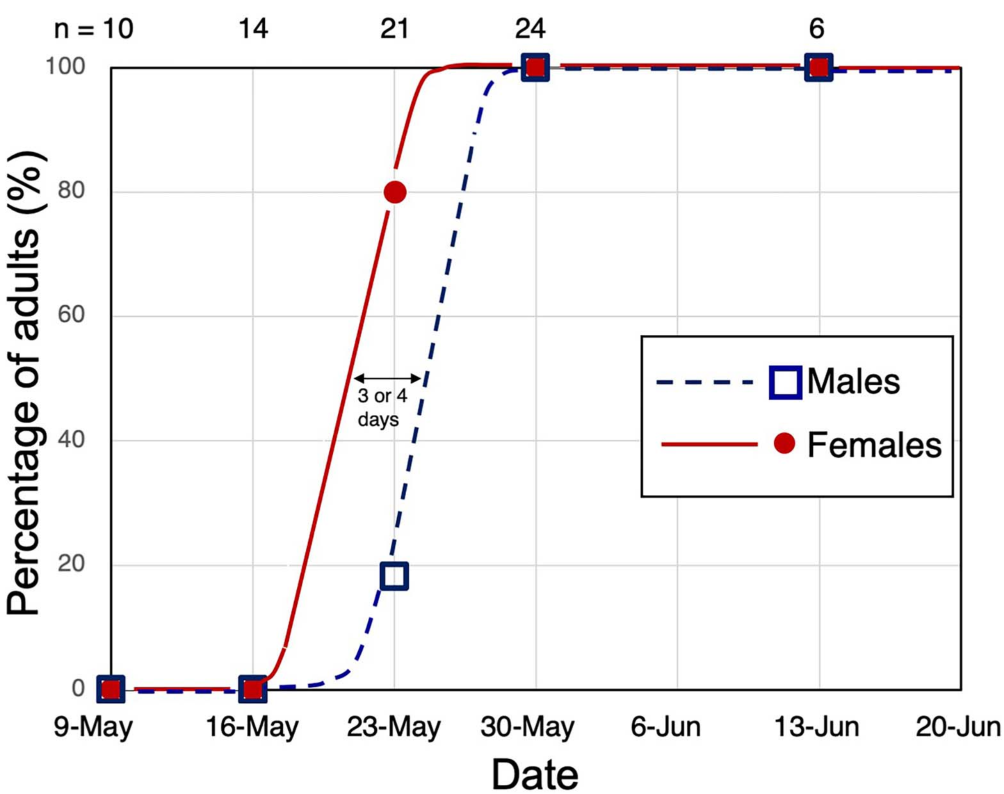

Figure 5.—Percentage of adults for females and males based on the same specimens used in Fig. 4 collected from May 9 to June 13. N at the top denote number of specimens. Note that females mature 3–4 days earlier than males. Unfortunately, only a single sample (23 May) contained both juveniles and adults. Moreover, the sample size is not large ($n = 21$). However, difference in the adult-juvenile ratio between the sexes in the May 23 sample was significant (chi square test, <0.005).

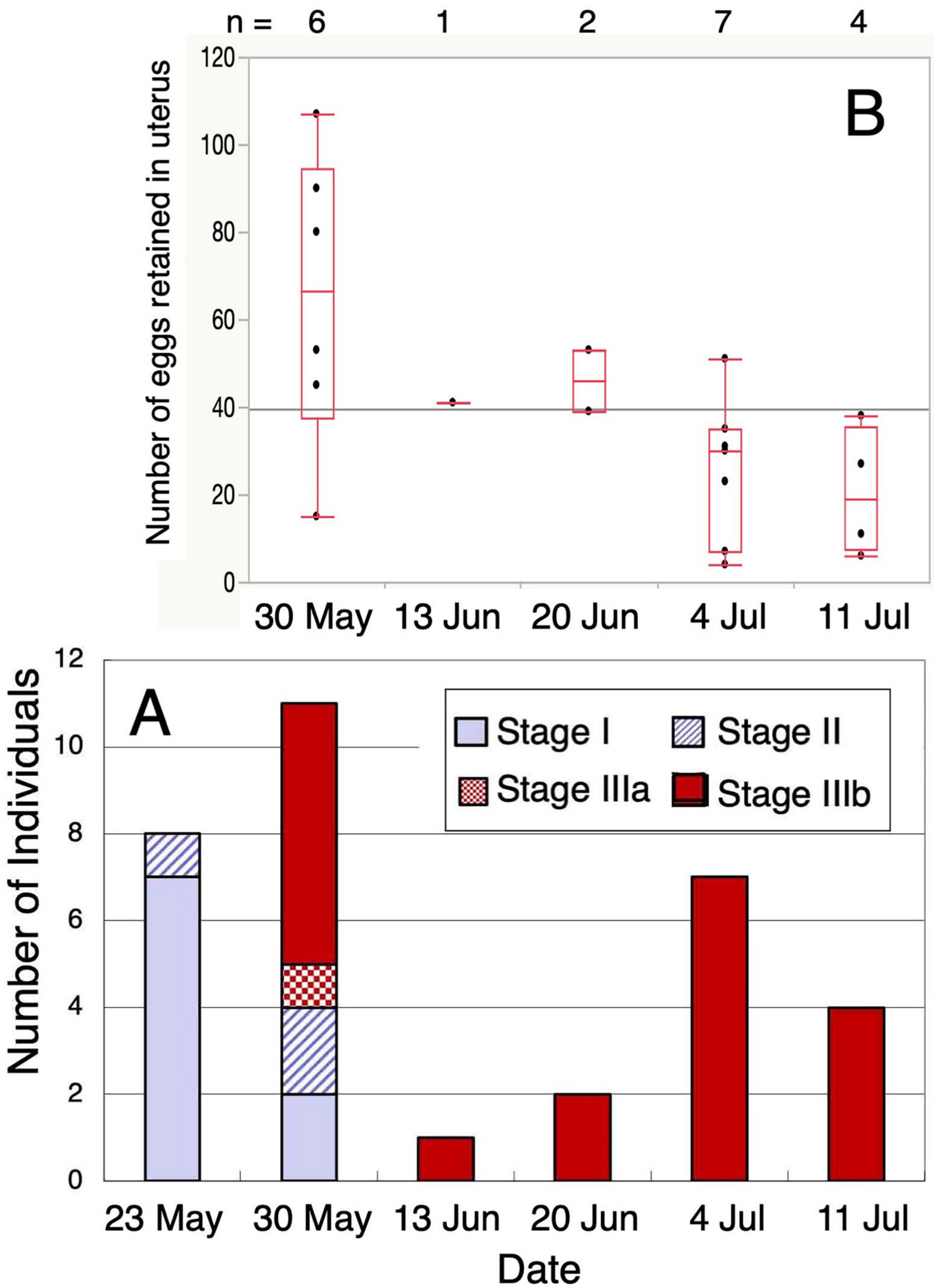

Figure 6.—Transition of ovarian development in adult females (A) and the number of eggs retained in the uterus internus in females in stage IIIb (B). Each box-and-whisker plot in B denotes inter-quartile range (box) with the central bar showing the median and total range (whiskers). A horizontal line starting from 40 on the Y axis represents the overall mean. Ns at the top are the number of specimens. Difference in the number of eggs among dates was significant (ANOVA, $P < 0.05$).

In *Paraumbogrella pumilio,* which overwinters as adults, adult males and females emerged at the same time in late July (Tsurusaki 2003). It is conceivable that this species does not show protogyny because the female's precedence over males does not make sense given that the reproductive season of the species starts after hibernation (Tsurusaki 2003).

Tsurusaki (2025) also suggested that elongated spermathecae may facilitate evolution of protogyny in *Odiellus aspersus* by enhancing last male sperm priority. *Himalphalangium spinulatum,* which is a univoltive species that overwinter as juveniles, also has an elongated spermatheca (see fig. 26D in Suzuki 1986). If elongation of the spermathecae would matter in evolution of the protogyny, it is likely that *H. spinulatum* also show protogyny with a larger time lag for the adult emergence between females and males. As expected, *H. spinulatum* showed a protogynous trend (Figs. 4–5), though the delay in reaching adulthood for males relative to females was rather

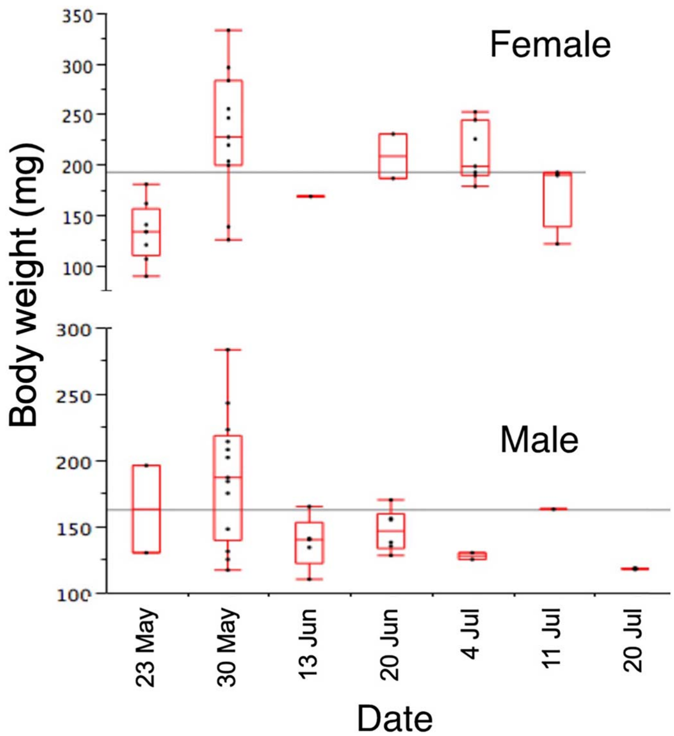

Figure 7.—Body weight of males and females (juveniles included). A horizontal line in each figure represents overall mean. Difference among dates were significant for both females and males (Kruskal-Wallis test, $P < 0.01$). Females seem to become heavier as their ovaries mature (cf. Fig. 6) and achieved highest weight on May 30. Males also attained highest body weight on May 30.

short (3–4 days) compared to that found in *Odiellus aspersus* (ca. 10 days: Tsurusaki 2025). It was found that the ovaries of females of this species rapidly mature and females contained matured eggs ready to oviposit in a rather short time (probably ca. a week) after the final molting (Fig. 6). We have no data on the ovarian development for other phalangiid species including *Odiellus aspersus* (Karsch, 1881) that show prominent protogyny. However, females of the two species of *Nelima* Roewer, 1910 (Sclerosomatidae) that also show

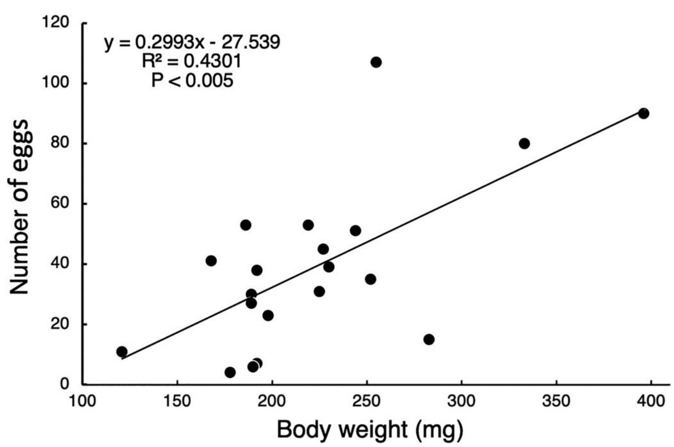

Figure 8.—The relationship between body weight and the number of eggs retained in the uterus internus in females in the ovarian stage IIIb.

protogyny seem to need 3 to 4 weeks for the maturation of ovary (cf. fig. 13 in Tsurusaki 2003). Both the number of matured eggs retained in the uterus internus and body weight of females of *H. spinulatum* were highest on May 30. It is likely that females become ready to mate with males and lay eggs in a considerably short time in this species. This feature may explain the rather short time lag (3–4 days) between females and males in maturation in this species, because males also have to become adults earlier to mate with females, even under the last male sperm priority hypothesis. In the future, it will be important to conduct further phenological study focusing on the onset of mating and oviposition, how many times females and males copulate with mates, and the dynamics of sperm competition in this species.

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