



Full length article

Swarming and mating behavior in *Ephemera orientalis* Mclachlan, 1875 (Ephemeroptera: Ephemeridae) with morphological analyses

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ABSTRACT

Swarming and mating behaviors of a mayfly species, *Ephemera orientalis* Mclachlan, 1875 were observed in 2015, 2016, and 2018 at a river bank of the Asahi River, Japan. Males started to make swarms between late April and middle May in 2016 and 2018. The numbers of mated pairs in a swarm correlated with the numbers of flying males in a swarm in 2016 and 2018. Swarms were formed during a limited period at dusk most probably because that interval is free from natural enemies. Males competed with each other to copulate with females in swarms. We clarified the function of the forelegs of males, which are significantly longer than those of females. Males used their forelegs to hold up a female from below. Besides forelegs, males have longer tails than females. We will discuss why sexual differences are found in these traits. Our results represent the first observation of swarm mating behavior in *E. orientalis*.

Introduction

Many animal species show species-specific mating systems, and they often form aggregations including leks and swarms to mate (Thornhill and Alcock, 1983). We call these aggregated flying males in sky a “swarm” (Thornhill and Alcock, 1983; Sivinski and Petersson, 1997). There are many observations of swarms in insect taxonomic groups including Ephemeroptera, Trichoptera, Hymenoptera, and Diptera (Sivinski and Petersson, 1997).

In some Ephemeroptera species, swarm behaviors have been recorded (e.g., Spieth, 1940; Thew, 1958; Britt, 1962; Savolainen, 1978; Harker, 1992; Francischetti et al., 2002; Peckarsky et al., 2002), and its seasonal occurrence depends on the emergence of adults. The emergence season of mayflies in Japan varies greatly depending on the species and region, from early spring (March) to late autumn (November). For example, adults of *Ephemera strigata* Eaton, 1892 form swarms once a year in Japan in May (Kuroda et al., 1984, Takemon, 1990). *Ephemera japonica* emerges from June to August, and emergence of *Ephemera orientalis*, which is the targeted species of the present study, is able to observe even in summer and autumn seasons (Maruyama and Hanada, 2016), but the mating behavior in swarms of *Ephemera orientalis* have not been investigated.

Ephemera orientalis Mclachlan, 1875, a common burrowing mayfly, is

distributed in temperate regions of East Asia including northeastern China, Mongolia, the Russian Far East, the Korean Peninsula, and the Japanese Islands (Hwang et al., 2008) including Hokkaido, Honshu, Shikoku, and Kyushu islands (Maruyama and Hanada, 2016). According to Maruyama and Hanada (2016) description, the larvae are found in flatland streams, and the adults emerge from May to November as described above. The adults are often attracted to lights (TM, personal observation). Adults emerge begins around sunset and fledges on the surface of the water.

However, the swarming and mating behavior of the species were not previously described. Thus, in the present study, we observed the swarming and mating behaviors of *Ephemera orientalis* along the river bank in Okayama, Japan. We also measured several morphological traits of males and females to find sexual dimorphisms in the species. Our results represent the first report of mating behavior and morphological analyses in *E. orientalis*.

Materials and methods

Observation was conducted at the Asahi River riverbank (north latitude 34.68, east longitude 133.94) in Okayama City during most of May in 2015 and 2016, and from April to June in 2018. The Asahi River is a river of special importance in Japan (like Class A river) flowing

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Fig. 1. View of the observation site of *Ephemera orientalis*.

through Okayama Prefecture, and the bottom of the river is pebbles and sand, so the observation was done in the middle stream area (Fig. 1). In 2017, we did not observe mayflies because of river construction, although this river work did not significantly change the flow of the river. Therefore, the 2018 survey was conducted on the opposite (=the other side) bank of the river. The environment on both sides of this river was almost identical, the river banks were covered with grasslands and a few trees. Although it has been reported that adults of this mayfly

emerge from May to November (Maruyama and Hanada 2016), in this study we counted adults only from mid-April to mid-May in both years (2016 and 2018). We only went to the river during these period.

Mating behavior was observed in May 2015. We counted the number of flying males and of mated pairs per 2 min and 4 min in 2016 and 2018, respectively. Three persons, who each held a paper ring (15 cm diameter) 10 cm in front of their eyes, stood apart every five meters on the riverbank. They counted the number of flying males and pairs in the ring visually. We started the observation at 18:00 (no mayflies start to fly at this time) and ended at 19:30 during 30th April and 14th May almost every day in 2016, and it started at 18:16 and ended 19:20 from 20th April to 14th May every day in 2018. To test for correlations between the maximum number of males and pairs (averaged value of three observers) in a day, we used analysis of variance (ANOVA) with number of pairs as the dependent variable, number of mayflies, year (2016, 2018), and interaction between number of mayflies and year as the explanatory variable. Information on weather during the observation periods were shown in Supplement Tabale 1.

In May 2016, we collected 68 male and 27 female flying adults using an insect net, and these were used for morphological analysis. Each individual was wrapped gently in a triangle parafilm and maintained at a deep freezer (−20 °C). The lengths of the body, abdomen, wing (right front wing), tail, and foreleg were measured using a Vernier caliper (Tajima, Tokyo). We used the analysis of covariance (MANOVA) with each morphological trait as a dependent variable, and sex and body length as explanatory variables to analyze differences between the sexes in morphological traits. All analyses were conducted by JMP Ver. 12.2 (SAS Institute Inc., 2015).

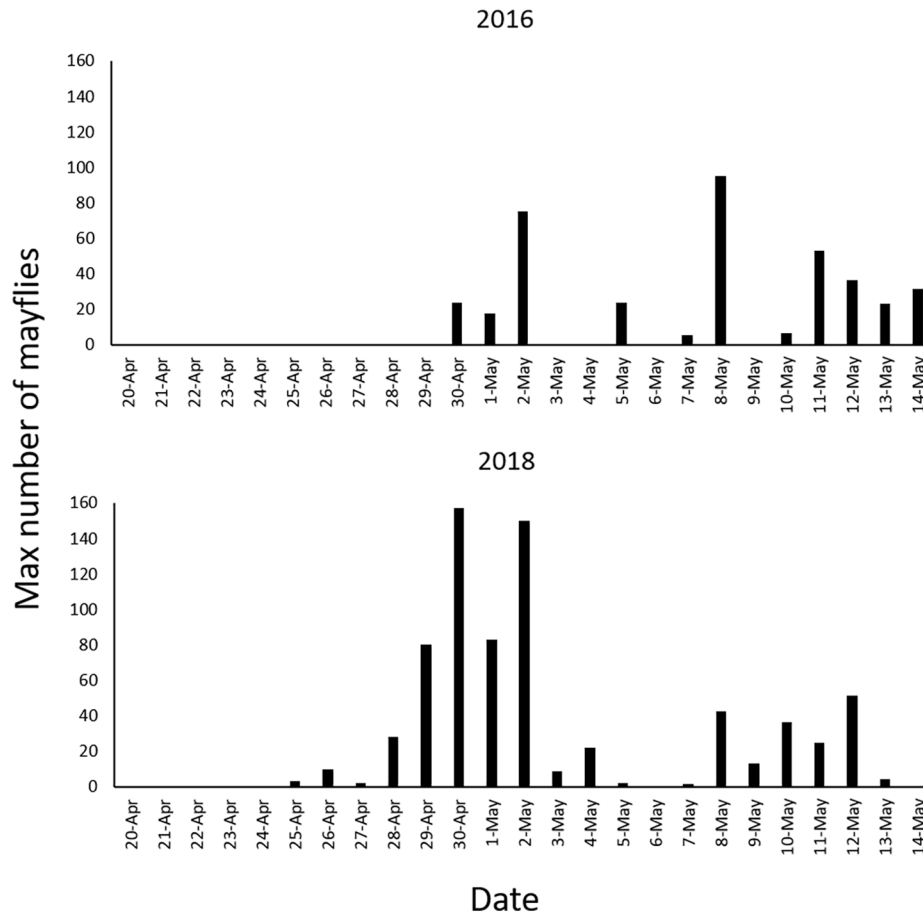


Fig. 2. Seasonal abundance of swarming males of *Ephemera orientalis* in 2016 (upper graph) and 2018 (lower graph). Each bar shows the average value (of three observation points) of maximum number of flying males per day.

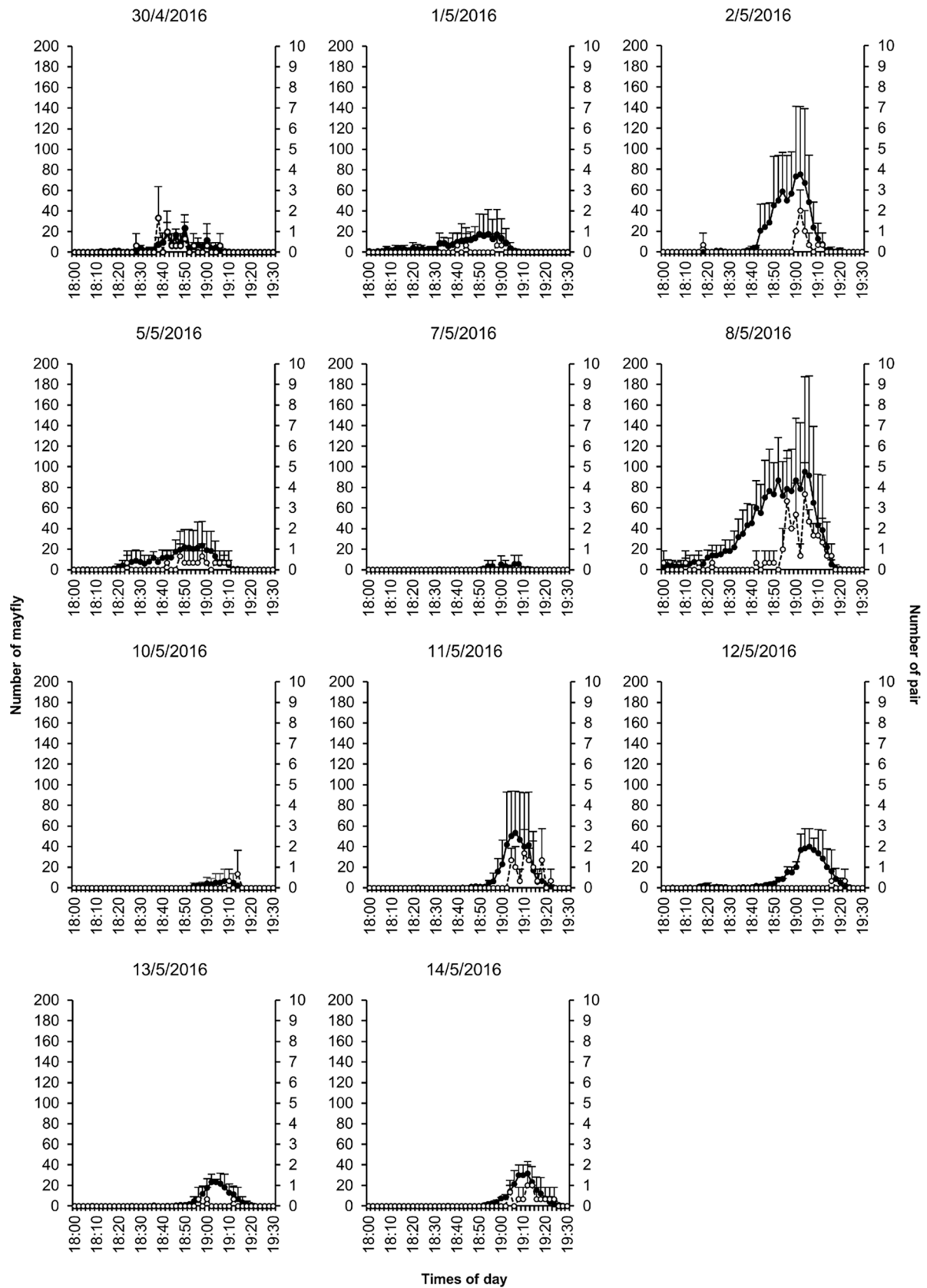


Fig. 3. Daily abundance of swarming males and mated pairs in 2016. Closed circles with a solid line and open circles with a dotted line show the average numbers with standard error as each bar of males and mated pairs, respectively.

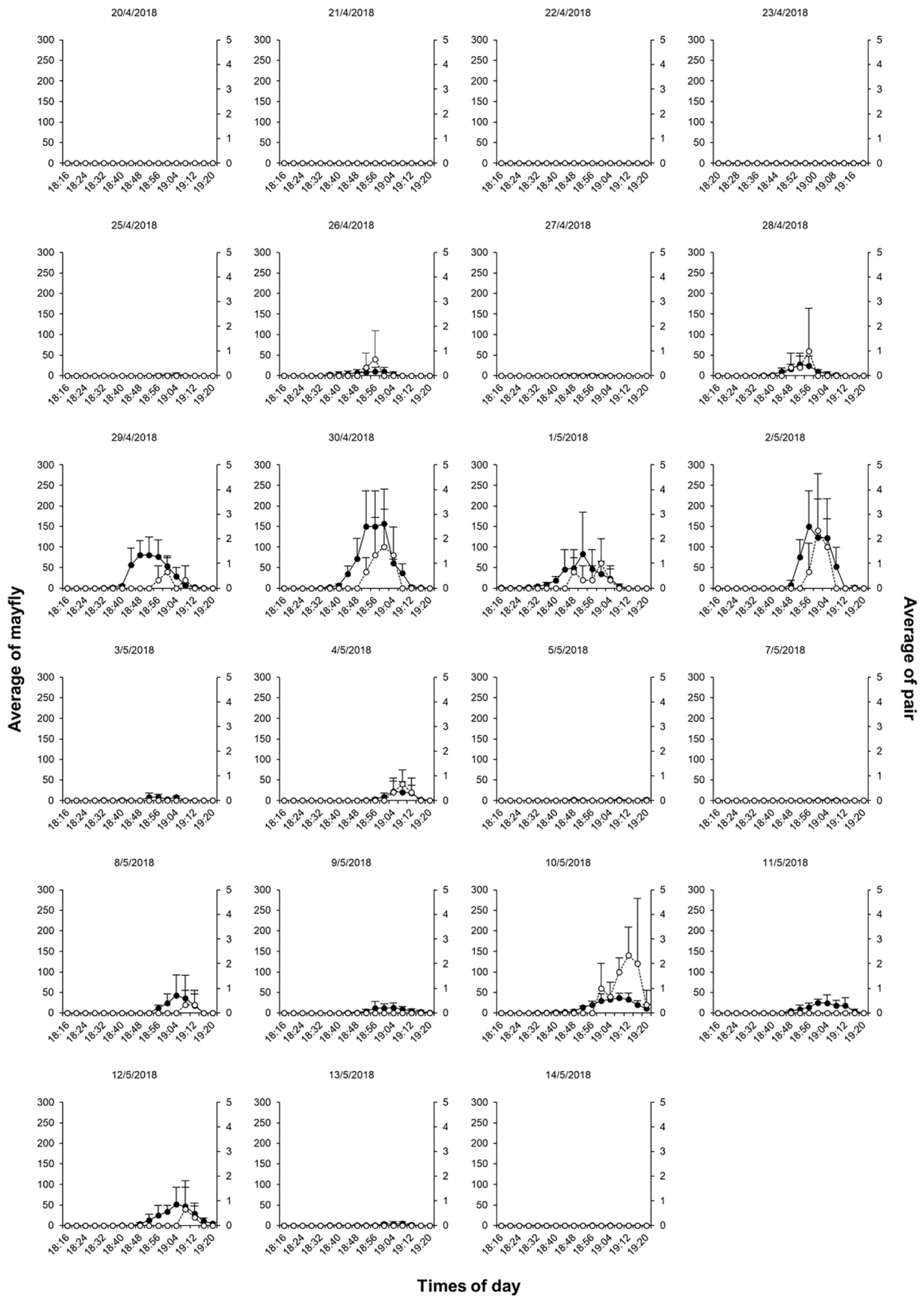


Fig. 4. Daily abundance of swarming males and mated pairs in 2018. Closed circles with a solid line and open circles with a dotted line show the average number with standard error as each bar of males and mated pairs, respectively.

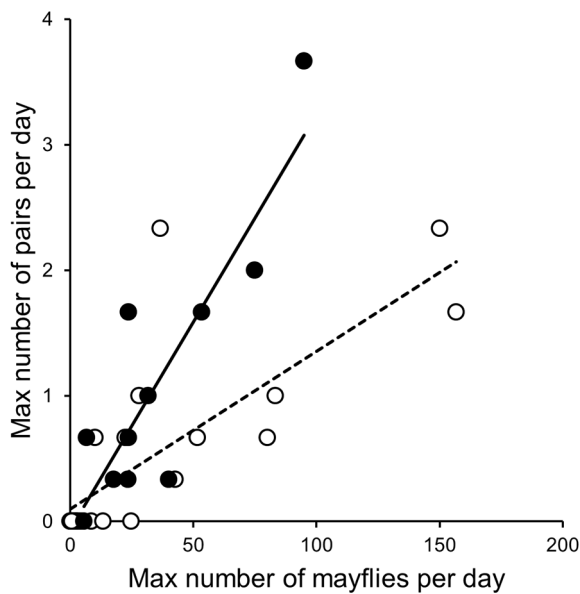


Fig. 5. Relationship between number of mayflies and mated pairs in 2016 (filled circle and solid line) and 2018 (open circle and dashed line), respectively.

Results and discussion

Video 1 shows flying males of *E. orientalis* in a swarm above the riverbank. Males who were resting on individual leaves of surrounding trees first flew away from the leaves, and started to form swarms. Later, females who were also resting on leaves of surrounding trees or grass on the river bank joined the swarms. In swarms, males tried to copulate with females. Sometimes, there were even several males trying to copulate with one female. Fig. 2 shows the seasonal abundance of flying males in 2016 (upper graph) and 2018 (lower graph). In 2016, we started observation from 30th April, and therefore did not have any information on the beginning of emergence. In 2016, the flying males were most abundant on 8th of May, and most abundant on 30th April in 2018. In 2018 we observed the whole period of spring and early summer, i.e., from April 20th May 14th, and swarming was first observed on April 25th and continued till May 13th.

Figs. 3 and 4 show the daily abundance of swarming adults and mated pairs in 2016 and 2018, respectively. During most of the observing days, many individuals of a sparrow species *Hirundo rustica* Linnaeus, 1758 started to fly from around 18:00 till 18:30, and many individuals of a bat species *Pipistrellus abramus* (Temminck, 1840) started to fly from around 19:15. During these periods, i.e., from 18:30 and 19:15, mayflies were most active, flew and made swarms at this time of day. We consider mayfly swarms can be formed during a limited period before sunset, namely at dusk, because of a possibility that the period is probably the least exposed to predation pressure by natural enemies. There was a gap between their activities which mayflies used to swarm, it might be a cause of lowering their exposure to predator risk. In other words, the main swarming might take place in the period of the decreased predator activity. However there may be other predators, and it is too early to determine the cause of the swarm being limited to this period. We need to observe the period of appearance of this mayfly species and predators at various locations in the future.

There were significantly positive correlations between swarm size and number of pairs in both 2016 and 2018 (Fig. 5, $F_{1,30} = 56.46$, $P < 0.0001$). The number of pairs was significantly higher in 2016 than in 2018 ($F_{1,30} = 7.68$, $P = 0.0095$), although the reason is unclear and remains to be inspected in future studies (see the data of weather conditions for the two years, although we could not have any conclusion because of the only two years). A significant interaction was also found



Fig. 6. An adult male of *Ephemera orientalis* with long forelegs.



Fig. 7. A mated pair in a swarm.



Fig. 8. A mated pair captured in an insect net.

between swarm size and year ($F_{1,30} = 11.40$, $P = 0.0021$), although the reason was not clear. It would be interesting to conduct an ecological study on the species, about its emergence period, differences between years in its beginning and end, as well as in differences in population densities in future.

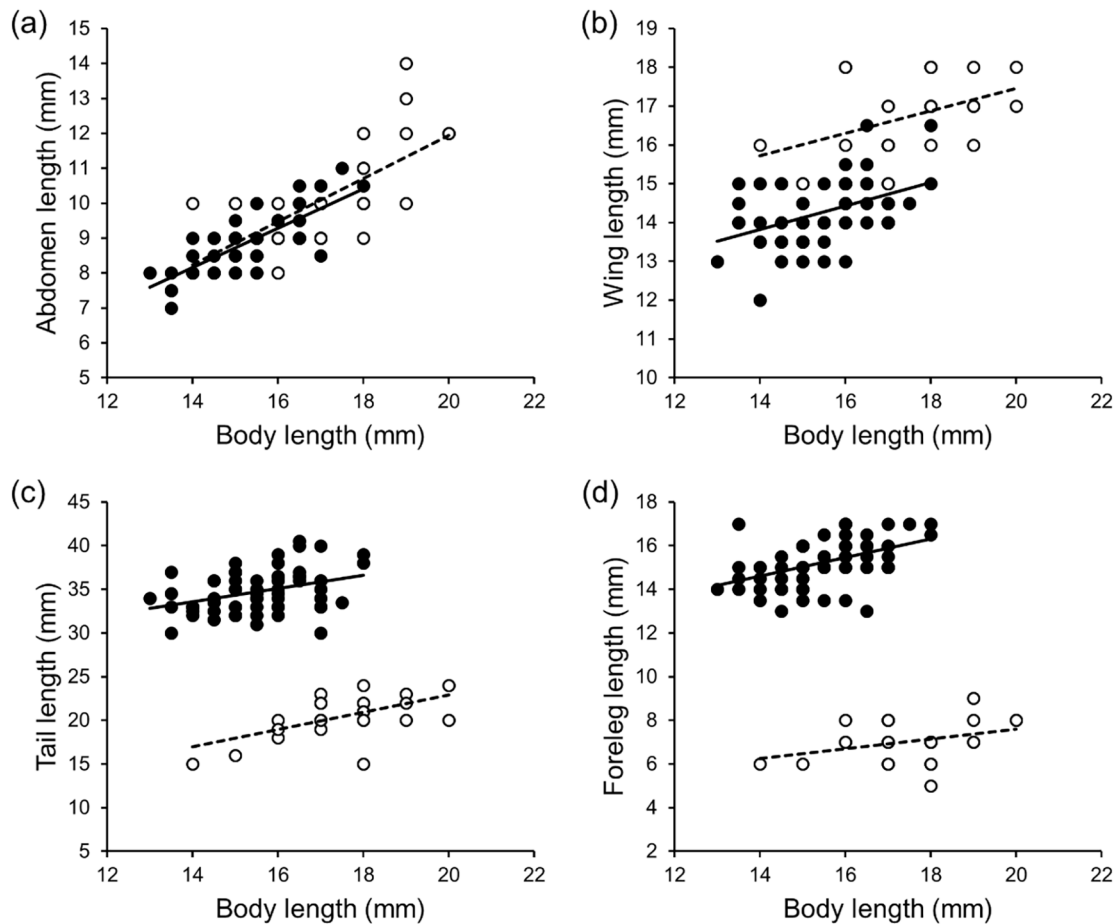


Fig. 9. Sexual differences in relationships between body length and some morphological traits including (a) abdomen length, (b) wing length, (c) tail length and (d) foreleg length. Closed circles with solid lines and open circles with dotted lines show males and females, respectively.

Table 1

Results of MANOVA for relationships between body length and some morphological traits with a sexual difference.

Trait	Factor	df	F	p
Abdomen length	Sex	1	1.1574	0.2848
	Body length	1	84.2763	<0.0001
	Error	92		
Tail length	Sex	1	649.2464	<0.0001
	Body length	1	20.2081	<0.0001
	Error	88		
Wing length	Sex	1	71.1192	<0.0001
	Body length	1	20.5026	<0.0001
	Error	92		
Foreleg length	Sex	1	1142.392	<0.0001
	Body length	1	20.0742	<0.0001
	Error	91		

Fig. 6 shows an adult male resting on a leaf of *Juglans mandshurica* Maxim. var. *sachalinensis*, (Komatsu) Kitamura, which is widely distributed along the riverbanks. As in all mayflies, males have very long paired forelegs. A flying male constantly searches for females who take off from the grass on the riverbank, and if a male finds a female, he tries to position himself under the female while flying (Fig. 7). Sometimes, a male competes with other several males in the sky to achieve this position. Finally, a male holds a female from below using these forelegs, as shown in Fig. 8.

Fig. 9 shows sexual differences in the relationships between body length and some morphological traits including lengths of (a) abdomen, (b) wing, (c) tail, and (d) foreleg. ANCOVA shows significant differences

between the sexes in tail length, wing length, and foreleg length (Table 1), while no significant difference was found between the sexes in abdomen length (Table 1).

The lengths of body and tail have been reported as about 20 mm and 35 mm, respectively (Maruyama and Hanada, 2016). These sizes are similar to the measurements of males in the present study (see Fig. 9). However, significant differences were found between the sexes in the lengths of wing tail and forelegs. Our observations clarified that males used the longer forelegs compared to females to hold a female in the air, while flying, and especially use the tarsi to sandwich the female thorax at mating (see Fig. 7). We often observed females flying with a male during copulation, and the male did not move his wings during mating, and only the female flapped her wings while they copulated (Fig. 7). Therefore, females may have longer wings than males for the flight to an egg-laying site, i.e., usually up-stream, while simultaneously carrying a copulating male. However, the wings of the female, which are larger than those of the male, may also be responsible for carrying the eggs. We need further experiments to prove these conjectures in the future.

Previous studies reported flexible life cycle for the species, depending on the habitat's location and environmental factors (e.g. Watanabe, 1992; Kuroda et al., 1984; Lee et al., 2008). Hwang et al. (2009) reported the species emerged in two occasions, in May-June, and August, from the Han River in Korea. In Japan, adults have been observed from May to November (Maruyama and Hanada, 2016). On the other hand, in the present study, we only counted the number of the adults from April to May because the flying individuals were not observed again till August (T.M. personal observation). Further observations are required during September and November in the future.

The present study is the first observation of a swarm mating system

observed in *Ephemera orientalis*, similar as in a relative species, *Ephemera strigata* (Kuroda et al., 1984, Takemon, 1990). Males swarmed on the riverbank, emerging females joined the swarm, and then males tried to mate with a female. The long forelegs of males were used to hold the females. Although we do not know why males form swarms for a limited period, cca. 40 min before dusk, a possible reason is to avoid attacks by natural enemies such as birds and bats.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aspen.2021.01.012>.

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