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Research Article

# Life cycles and larval and pupal habitats of eleven Japanese *Rhyacophila* (Trichoptera) species at the mountain streams in central Honshu*

Akikazu Taira[1,2]

1 *Faculty of Liberal Art and Sciences, Osaka Metropolitan University, Gakuen-cho 1-1, Naka-ku, Sakai, Osaka 599-8531, Japan*
2 *Osaka Museum of Natural History, Nagai Park 1-23, Higashi-sumiyoshi, Osaka, 546-0034, Japan*

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Corresponding author: Akikazu Taira (akikazu-t@ge.osaka-sandai.ac.jp)

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## Abstract

The life cycles and microhabitats involved in aquatic stages of eleven *Rhyacophila* Stephens, 1836 species living in two neighboring Japanese mountain streams in Nara Prefecture, central Honshu, were investigated. Eight species had univoltine life cycles; seven species had spring to early summer emergence seasons. One species had an autumnal emergence. Four univoltine species had periods where no larvae or pupae were collected in aquatic stages, possibly a period of egg diapause. Three species had bivoltine life cycles, with early summer and autumn emergence seasons. *Rhyacophila nipponica* Iwata, 1927, *R. manuleata* AV Martynov, 1934, and *R. shikotsuensis* Iwata, 1927, in the *nigrocephala* species group Ross, 1956, have larvae which spend most of their larval stage in the hyporheos. The larvae of *R. transquilla* Tsuda, 1940, also inhabit the hyporheic zone, although the vertical microhabitat used by this species differed from the former three species. All four of these species have hyporheic larval stages, but in these species the first and final instar larvae reside on the surfaces of cobbles. The first instar larvae seem to live on stone surfaces immediately after hatching, and subsequently move to the interstices of sand. Final instar larvae live in the interstices of sandy bottom, and move to stone surfaces for pupation. Eleven species of the genus *Rhyacophila* had diverse life histories, microhabitat preferences, and experienced differing water temperature regimes during larval stages at the sites. Life history and habitat preference variation enhances the species richness of the genus and coexistence of species in upper stream of the Kinokawa River.

## Key Words

coexistence of species, egg dormancy, hyporheic zone, microhabitat shift, *nigrocephala* species group, species richness

## Introduction

*Rhyacophila* Stephens, 1836 is one of the most species-rich genera of Trichoptera of Japan. *Rhyacophila* larvae are rarely numerically dominant in Japanese streams, but they are common and diverse in some stream habitats in Japan. In the Kinokawa River, which flows from east to west in the northern part of the Kii Peninsula on Honshu Island, numerous *Rhyacophila* species occur in the upper reaches of the river, with as many as 16 species at one site (Tanida et al. 1995; Nakano and Tanida 1998; Taira and Tanida 2011). There are a few limited ecological studies on the Japanese species of *Rhyacophila*. Taira and Tanida (2011) investigated *Rhyacophila* habitat preferences in two Honshu Island streams and found that while most *Rhyacophila* larvae inhabit stone surfaces of the riverbed, larvae of the *nigrocephala* species group Ross, 1956 live in the interstices of the riverbed sediments. The complete life histories of only five species have been elucidated from larval and adult studies (Ito 1999; Taira et al. 2013; Taira and Tanida 2014; Aoya and Tanida 2023), and the behaviors of only nine of the 57 species in Japan have been studied (Taira and Nozaki 2020).

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In this study, I investigate the life cycles and microhabitats of eleven *Rhyacophila* species that inhabit the upper and middle reaches of some Japanese rivers, and clarify the life cycles and microhabitat preferences of those *Rhyacophila* species.

# Method

## Sampling sites

I selected two study sites from the headwater tributaries of the Kinokawa River, Nara Prefecture, central Honshu, Japan (Fig. 1). Although those two sites were geographically close and only separated by flow distance of 6 km, the altitude, river order, river width, etc. of these river sites are quite different. The upper study site at the Omata Stream (Site 1: 34.3769°N, 136.0516°E, 500 m a.s.l.) is a second-order stream, 5 m width and mean slope of 0.074 (Fig. 2). The lower study site, at the Shigo Stream (Site 2: 34.3782°N, 136.0047°E, 345 m a.s.l.), was is a third-order stream, 15 m width and mean slope of 0.012 (Fig. 2). From reaches at and adjacent to these two sites, 16 species of *Rhyacophila* have been collected (Tanida et al. 1995; Nakano and Tanida 1998; Taira and Tanida 2011). The watersheds of those two streams are extensively forested with Japanese cedar *Cryptomeria japonica* (L.f.) D.Don, 1841. However, the riparian zones of the streams are well covered with secondary deciduous vegetation by species of an *Alnus japonica* (Thunb.) Steud, 1840 and oak, *Qurercus serrata* Murray, (1784) and by some maple species, *Acer* L. 1753 spp.

## Species group and taxonomic description of Japanese *Rhyacophila* species

The targets of this investigation were *nigrocephala* species group and *retracta* species group Ross, 1956. These groups were defined by Ross (1956) based on the morphological characteristics of male genitalia.

*Nigrocephala* species group is confined to the region from India or Himalaya to Japan and consists of

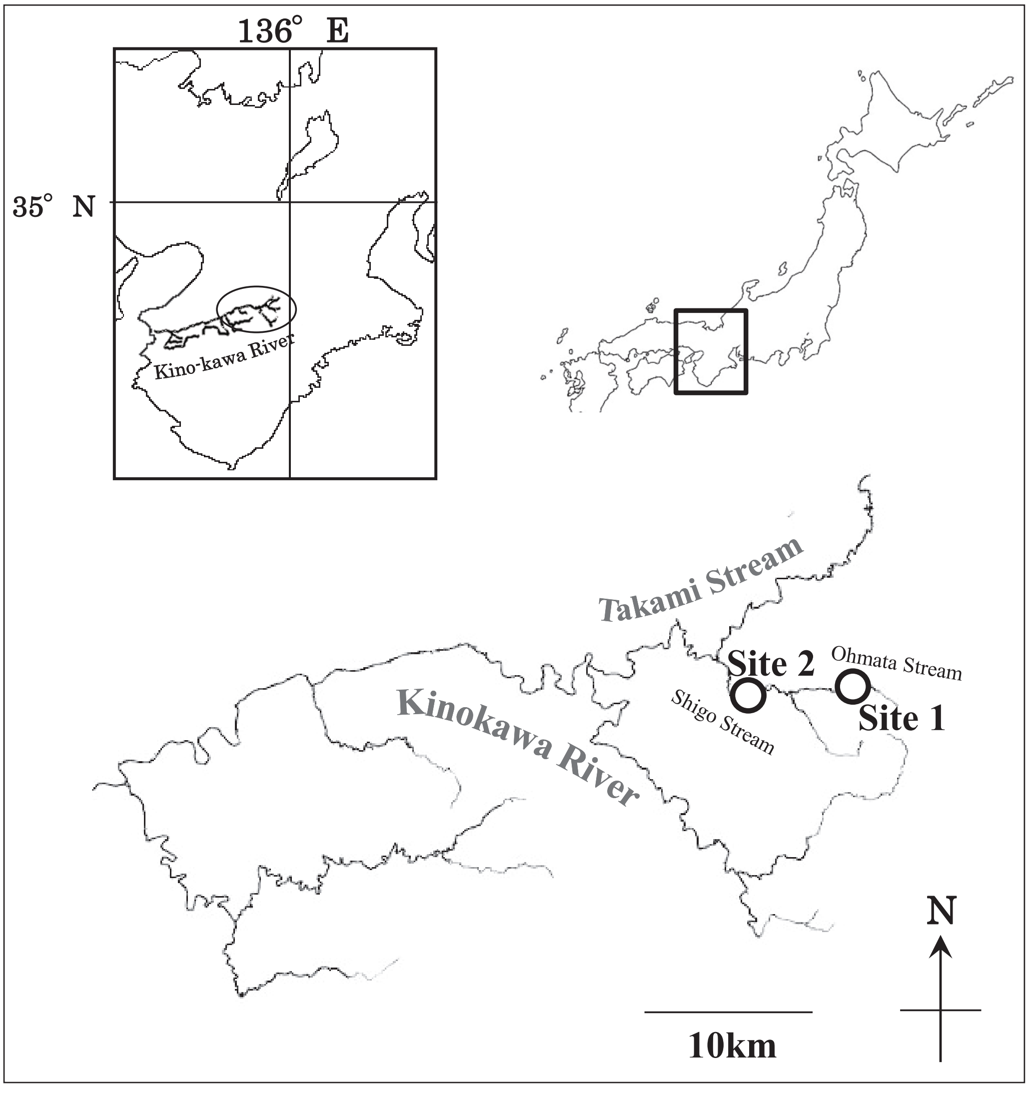

**Figure 1.** Two sites selected for the life cycle study of *Rhyacophila* species, located in the Kinokawa River, Nara Prefecture, Nanataki (Site 1) and Sebato (Site 2).

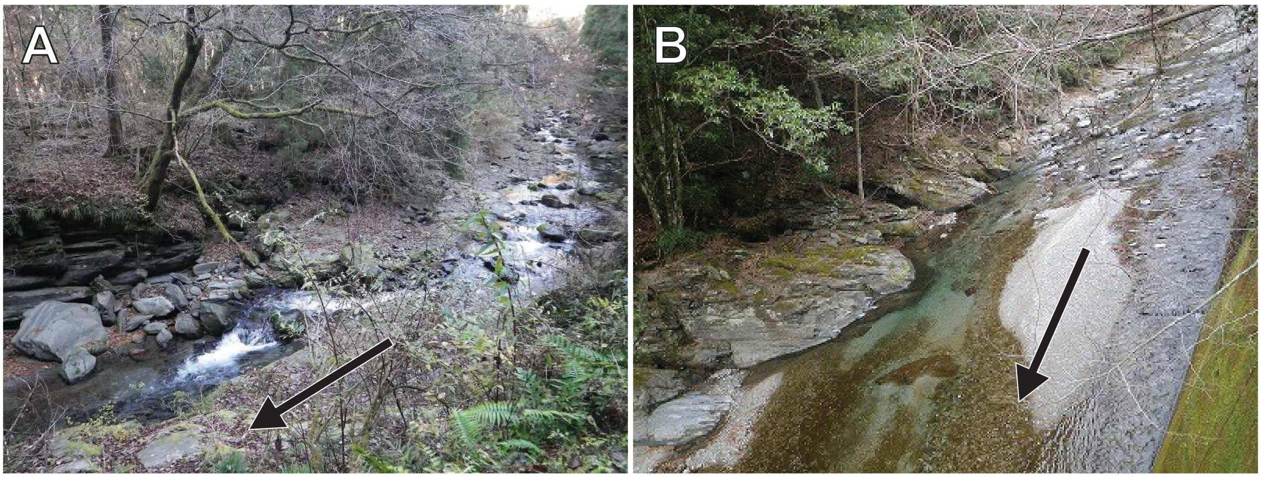

**Figure 2.** Study sites at Site 1 (**A**) at Ohmata Stream and Site 2 (**B**) at Shigo Stream. Arrows indicate flow directions.

32 nominal species (Schmid 1970). There are six valid species in Japan (Kuranishi 1999; Hattori 2005); the targets in this study were *R. nipponica* Navas, 1933, *R. shikotsuensis* Iwata, 1927, *R. kawamurae*, Tsuda, 1940. However, *R. kawamurae* was detected as a synonym of *R. manuleata* AV Martynov, 1934 (Malicky 2014). Therefore, I referred *R. kawamurae* to as *R. manuleata* in this study. Malichy (2014) mentions *R. shikotsuensis* as a synonym for *R. brevicephala* Iwata, 1927; these two species commonly inhabit rivers in Japan. However, the morphological characteristics of male genitalia and larva are very different between these two species (Hattori 2005), and Kuranishi (1999) and Hattori (2005) reported *R. brevicephala* as a completely different *brevicephala* species group Kuranishi, 1999, not as *nigrocephala* species group. Therefore, I showed this species as *R. shikotsuensis* in this study.

*Retracta* species group is confined to the region from Siberia to Japan and consists of four nominal species (Hattori 2005). The targets in this study were *R. motakanta* Schmid, 1970 and *R. orthacanta* Emoto, 1979. *Rhyacophila* sp. RC was distinguished only by larval stage, which includes several species of the *retracta* species group (Hattori 2005). Four species have been assigned to this species group. *Rhyacophila retracta* Martynov, 1914, distributed from Siberia to Japan. The other three species, *R. lambakanta* Schmid, 1970, *R. orthacantha* Emoto, 1979, *R. motakanta*, are currently known only from Japan. Among this species group, adults of *R. orthacantha* were collected only from Site 1 and adults of *R. motakanta* only from Site 2. Therefore, I concluded larvae of *R.* sp. RC to be *R. orthacantha* in Site 1 and *R. motakanta* in Site 2.

## Water temperature measurement

The stream water temperatures at the two sites were measured using temperature data loggers (Thermoclon type G, KN laboratories, Osaka, Japan) at one-hour intervals during the period from September 2016 to September 2017, with a unit resolution of 0.1 °C (Fig. 3). Mountaineering harkens were inserted into cracks in the exposed rock surfaces on the riverbank, to fix the data loggers. Piano wires with a diameter of 1.5 mm were passed through the hole of the harkens, and carabiners were used to connect the data logger and their protective vinyl chloride pipe. Data loggers were submerged under water and covered by several stones on the loggers to prevent disruption to measurements.

## Collecting samples

Life cycles and microhabitat usage by larvae of *Rhyacophila* were investigated monthly over the course of approximately one year (September 27, 2016 to September 12, 2017) at the two sites. To sample larvae and pupae, I used the same method reported by Taira (2018): a stream-bed area of 0.25 × 0.25 m is defined as one sample. At each site, 30 samples were collected from rapids covered with cobble and gravel, and 30 samples from runs covered with gravel and sand. A total of 60 samples were collected at each event at each site. In order to examine the microhabitat of larvae and pupae in rapids, samples were collected from two vertical sections of the stream-bed: cobbles on the stream-bed (hereafter “surfaces of cobbles” as the microhabitat) and interstices of gravel and sand under cobbles (hereafter “interstices of sand” as the microhabitat). In the run, samples were collected from one vertical section from interstices of gravel and sand. Adults of *Rhyacophila* species were collected at each sampling day from September to November in 2016, April to November in 2017 by two methods: net sweepings were conducted for two hours before sunset, and adult collecting using pan traps illuminated with a fluorescent black light deployed from sunset to sunrise.

In the field, specimens of larvae, pupae, and adults were preserved in 70% ethyl alcohol at each site. In the laboratory, using the stereoscopic microscope (magnification: 10–40×, SMZ-10, Nikon, Tokyo, Japan), larvae, pupae, and adults were identified to species following Hattori (2005).

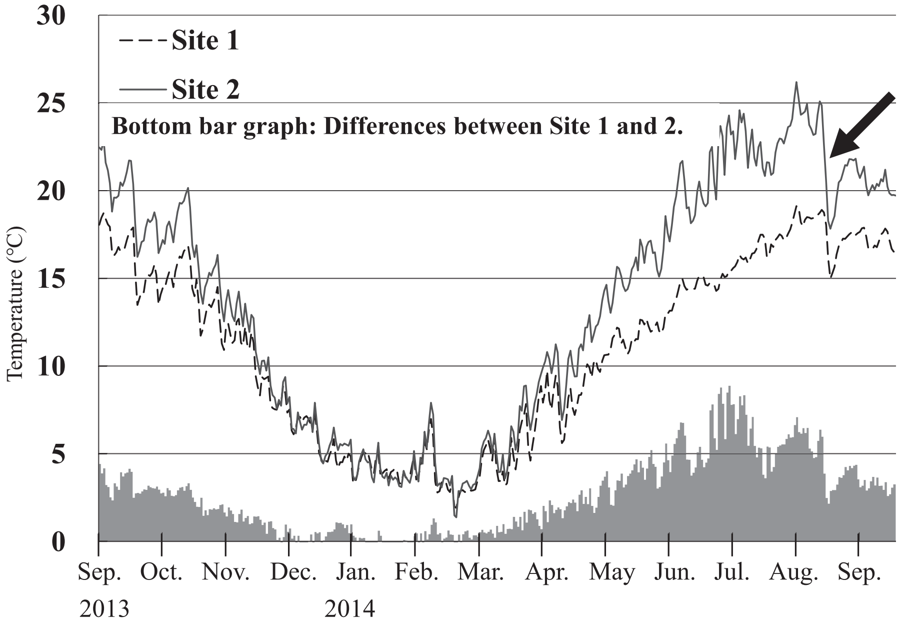

**Figure 3.** Temporal changes of daily average water temperatures at Site 1 and 2 from September 2013 to September 2014. Arrows indicate a significant decline in water temperature.

## Larval instar determination

Head width ranges for each instar were determined from the frequency distributions of the measured sizes. Larval heads were cut from the bodies and photos captured at scale (10 mm) using a digital camera (OPTIO W90, PENTAX, Tokyo, Japan) under the stereoscopic microscope. After uploading those photos into the personal computer (PC-GL17MG198, NEC, Tokyo, Japan), the head capsule width at the widest axis was measured to the nearest 0.001 mm using the image software (Image J, National Institutes of Health, Maryland, USA). Frequency distribution of head widths for each species was represented using a histogram with 0.02 mm intervals for those less than 0.42 mm and 0.04 mm intervals for those greater than 0.42 mm. For all species, five peaks were clearly recognized (Fig. 4). There was no overlap of the head widths between larval instars within each species.

## Investigation of microhabitat shift with growth and development

For the species collected from two types microhabitats at rapids (surfaces of cobbles and interstices of sand), i.e., Rhyacophila manuleata, *R. shikotsuensis*, *R. nipponica*, and *R. transquilla* Tsuda, 1940, I clarified larval habitat shift with the growth of wet weight and instars.

I selected instars larvae, first and fifth instar, with those collected from both microhabitats, the wet weights of that instar larvae were measured. For those larva, excess water on the body surface was thoroughly wiped off with a paper towel, and the measurement of weight was performed with an accuracy of up to 0.01 mg using an electronic balance (AG135, Mettler Toledo, Columbus, USA).

Wet weights of those larvae were compared between two microhabitats using the Mann-Whitney U test (excluding when the number of collected individuals was small). If there was a significant difference in larval weight between those microhabitats, I concluded that the larvae shift from the microhabitat of the light larva to another microhabitat of the heavy larvae.

# Results

## Seasonal change of stream temperatures at the two sites

Site 1 and 2 have a 55 m difference in elevation, although water temperatures were on roughly similar trajectories during winter and spring periods of the year (November to April) (Fig. 3). From summer to autumn (May to October), however, the water temperature trajectories diverged, where Site 2 was frequently 2–8 degrees warmer than Site 1. A significant decline in water temperature was observed on August 11 at both sites (Fig. 3, arrow) with heavy rain on August 9 (total rainfall: 262 mm) and August 10 (total rainfall: 106 mm) (Japan Meteorological Agency 2023).

## Species composition of *Rhyacophila* at the two sites

The larvae of eleven species of *Rhyacophila* were collected in this study: nine species from Site 1 and ten species at Site 2 (Table 1). Seven species, i.e., *R. lezeyi* Navás, 1933, *R. manuleata*, *R. shikotsuensis*, *R. nipponica*, *R. transquilla*, *R. kisoensis* Tsuda, 1940 and *R. brevicephala*, were common in both Site 1 and 2. Two species, *R. orthacantha*

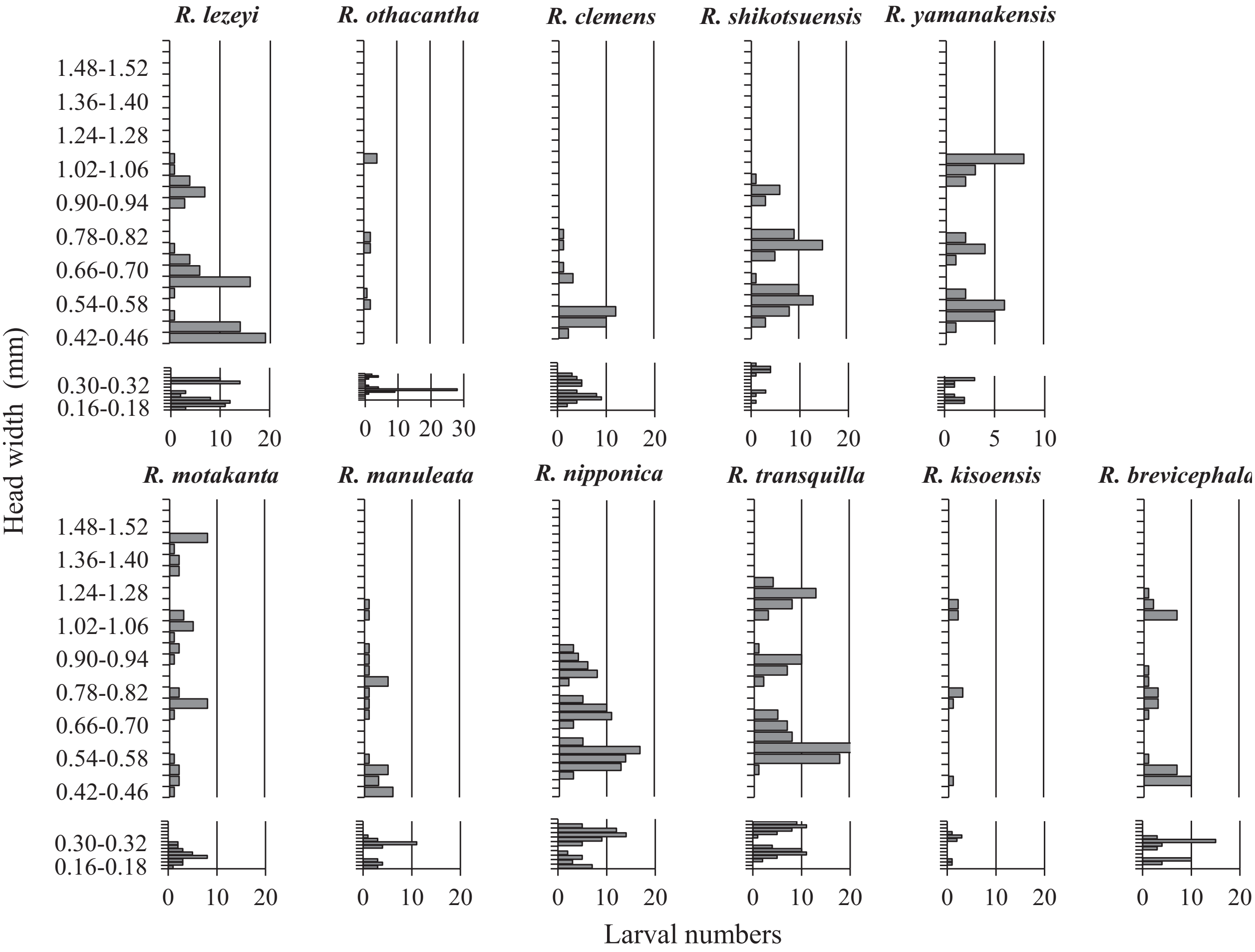

**Figure 4.** The head capsule width of eleven *Rhyacophila* species of larvae.

**Table 1.** Collected numbers of *Rhyacophila* larvae at Site 1 and Site 2.

| | | 2013 | | | | 2014 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 27 Sep. | 20 Oct. | 5 Nov. | 6 Des. | 17 Jan. | 13 Feb. | 22 Mar. | 20 Apr. | 18 May. | 15 Jun. | 6 Jul. | 1 Aug. | 12 Sep. |
| Site 1 | ***R. lezeyi*** | **7** | **3** | **45** | **11** | **30** | **18** | **11** | **5** | **17** | **4** | | | **2** |
| | ***R.* sp. RC (*R. orthacantha*)*** | **1** | **3** | **2** | **4** | **3** | **2** | **4** | **2** | **2** | **30** | **3** | **3** | **4** |
| | ***R. clemens*** | **3** | **15** | **9** | **12** | **5** | **4** | **5** | **7** | | **2** | **2** | **4** | **5** |
| | *R. manuleata*** | | 3 | 1 | 1 | 3 | 2 | | | 3 | 1 | | | |
| | ***R. shikotsuensis*** | **29** | **6** | **3** | | | | | | **4** | **7** | **15** | **27** | **10** |
| | *R. nipponica* | | | 2 | | 3 | 9 | 1 | | 2 | 3 | | | |
| | *R. transquilla* | | 18 | 1 | 10 | 4 | 2 | | | | | | 4 | 3 |
| | *R. kisoensis* | | | | 2 | | | 2 | | 4 | 2 | | | |
| | *R. brevicephala* | | | | 2 | | | | | 2 | 3 | | | |
| Site 2 | *R. lezeyi* | | | | | | 1 | | | | | | | |
| | ***R. yamanakensis*** | **2** | | **1** | | | **4** | **4** | **2** | **4** | **11** | **15** | **3** | |
| | ***R.* sp. RC (*R. motakanta*)*** | **1** | **1** | **2** | **2** | **8** | **16** | **16** | **9** | | **17** | **5** | **4** | **1** |
| | ***R. manuleata***** | | **5** | **7** | **19** | **4** | **13** | **10** | **3** | | | | | **7** |
| | *R. shikotsuensis* | | | | | | | | | | 3 | 2 | 7 | |
| | *R. nigrocephala* | 2 | | | | 4 | 2 | 1 | 4 | 1 | | 2 | 1 | |
| | ***R. nipponica*** | **19** | **2** | | **1** | **55** | **13** | **4** | **25** | **8** | **13** | **14** | **20** | **5** |
| | ***R. transquilla*** | | **6** | **54** | **67** | **27** | **12** | **18** | **10** | | | | | **12** |
| | ***R. kisoensis*** | | **2** | **7** | **3** | **1** | **2** | **2** | **8** | | | | | |
| | ***R. brevicephala*** | **2** | | **1** | **2** | **8** | **11** | **6** | **16** | **3** | **5** | **10** | **6** | **10** |

* *Rhyacophila* sp. RC distinguished by include several species of the *Retracta* species group (Hattori 2005). I conclude that *R.* sp. RC in Site 1 as *R. orthacantha* and in Site 2 as *R. motakanta*, since other adults of *Retracta* group were not collected from the study site.

** *Rhyacophila kawamurae* was detected as a synonym of *R. manuleata* (Malicky 2014). Therefore, I referred to *R. kawamurae* as *R. manuleata* in this study.

and *R. clemens* Tsuda, 1940, were collected only from Site 1, and three species, *R. yamanakensis* Iwata, 1927, *R. motakanta*, and *R. nigrocephala* Iwata, 1927 were collected only at Site. 2.

## Life cycles and microhabitat shift of *Rhyacophila* species

### ***Rhyacophila lezeyi*** (Site 1)

This species was mainly collected at Site 1 and was the most dominant *Rhyacophila* species at the site, but only one larva was collected at Site 2 (Table 1). The adults of this species were collected from June to August 2014 (summer season) (Fig. 5).

The first instar larvae appeared in September 2013 (and 2014), and were continuously collected until January 2014. The first to fourth instar larvae were collected in November and December 2013, and no clear peaks of larval stages (instars) were detected in these months. The fifth instar larvae appeared from January to June 2014, but a few first instar larvae still remained in that period. The peaks of the third instar larvae were observed in January and February 2014, and the peak of the fourth instar larvae was in March 2014. The fifth instar larvae predominated in April and pupae in May and June 2014, but the fourth and fifth instar larvae still remained in these months.

I infer that this species had a univoltine life cycle with summer emergence season (Fig. 5). However, the dispersion of developmental stages (instars) was wide, especially in the winter season (from November to March). The clear development of instars was observed even in winter (from November to January). Development decreased from January to February, when the daily mean temperature was less than 4 °C (Fig. 3). The clear development was recovered after March.

All larvae and pupae were collected only from surfaces of cobbles (Fig. 5), and no microhabitat shift was confirmed within their aquatic stage.

### ***Rhyacophila orthacantha*** (Site 1)

The adults of this species were collected at Site 1 from May to July 2014 (Table 1, Fig. 5). The first instar larvae

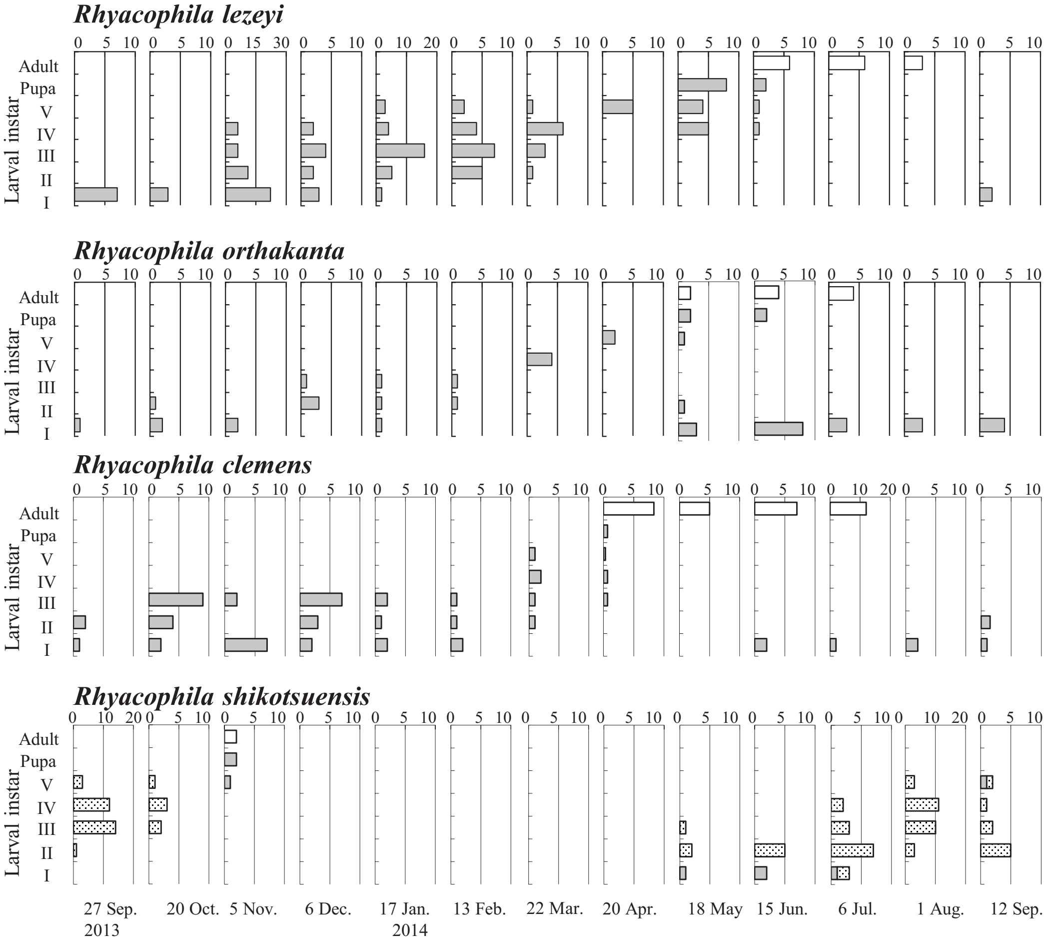

**Figure 5.** Life cycle patterns of four *Rhyacophila* species at Site 1 showing the frequency of larval instars and pupae collected from each of the 30 quadrat samples (250 × 250 mm) from two types of streambeds; adults were collected by net sweeping and pan traps with black lights. In white rectangles (black border): adults collected by pan traps or by an entomological net. In grey rectangles: larvae and pupae collected from the surfaces of cobbles. In dotted rectangles: larvae and pupae collected from the interstices of gravel and sand.

appeared from September to November 2013 and May to September 2014 and I infer that they were continuously collected for seven months until November. The second and third instar larvae were collected in December 2013. The peaks of the fourth instar larvae were observed in March, and the fifth instar larvae predominant in April 2014. The pupae appeared in May and June 2014.

This species had a univoltine life cycle with early summer emergence season (Fig. 5). The clear development of larvae was observed even in the winter to spring season (January to April), but the development of first instar larvae was hardly observed from May to November (Fig. 5), although the daily mean temperature in the period exceeded 12 °C (Fig. 3).

All larvae and pupae were collected only from the surfaces of cobbles (Fig. 5), and no microhabitat shift was confirmed within their aquatic stage.

### ***Rhyacophila clemens*** **(Site 1)**

The adults of this species were only collected at Site 1 from April to July 2014 (spring season) (Table 1, Fig. 5). The first instar larvae appeared in June 2014, and was the only instar collected for three months until August 2014. The first and second instar larvae appeared in September 2013 and 2014. No clear peaks of instars were detected during October 2013 to February 2014, when larvae were collected across the range of first to third instars. Fourth and fifth instar larvae first appeared in March, but second and third instar larvae persisted into April, even as pupae appeared.

This species had a univoltine life cycle with early spring to early summer emergence season (Fig. 5) The clear development was observed even in the winter to spring season (February to April). But the development of first to third instar larvae were hardly observed from December to February, when the water temperature was below 5 °C (Fig. 3).

All larvae and pupae were collected only from the surfaces of cobbles (Fig. 5), and no microhabitat shift was confirmed within their aquatic stage.

### ***Rhyacophila shikotsuensis*** **(Site 1)**

This species appeared at both sites, but more numerous larvae were collected at Site 1 (Table 1). At Site 2, only a few larvae were collected from June to August 2014. At Site 1, the adults and pupae of this species were collected in November 2013 (autumn season) (Fig. 5). The first to third instar larvae appeared in May 2014, the fourth instar larvae were collected in July 2014, and the fifth instar larvae were collected in August 2014.

This species had a univoltine life cycle (Fig. 5). But unlike other univoltine species, the emergence season was in autumn. A clear development of instars was observed from summer to autumn (June to October, Fig. 5) with daily mean temperature from 15 to 20 °C (Fig. 3). There was a period without collected larvae and pupae; pupae (December to April) and adult were collected from early winter (November) (Fig. 5), when the daily mean temperatures were 9.4 °C (Fig. 3).

For the microhabitat preferences, all second to fourth instar larvae were collected from interstices of sand (Fig. 5). However, the first and fifth instar larvae were collected from both surfaces of cobbles and interstices of sand, and all pupae were collected only from surfaces of cobbles. The first instars were collected from surfaces of cobbles in May 2014. But in June 2014, some first instar larvae appeared also in interstices of sand. I compared the wet weight of first instar larvae between those two occasions. The wet weights of the first instar larvae in interstices of sand were significantly heavier than those of the larvae in surfaces of cobbles (Mann-Whitney U test: $P < 0.05$, Fig. 8). It was suggested that the first instar larvae shifted their microhabitat from surfaces of cobbles to interstices of sand.

The fifth instar larvae began to appear from August 2014 and were all collected from interstices of sand (Fig. 5). In September 2014, however, the fifth instar larvae were collected also from surfaces of cobbles. The wet weight of fifth instars' larvae on surfaces of cobbles was heavier than that interstices of sand (Fig. 8). I infer that the fifth instar larvae may shift from interstices of sand to surfaces of cobbles, before pupation, contrary to the first instar larvae.

### ***Rhyacophila yamanakensis*** **(Site 2)**

This species was collected only at Site 2 (Table 1). The adults of this species were collected from June to July and September, thus there might exist two generations (Fig. 6).

The first generation (wintering generation) appeared from March to July 2014, the first and second instar larvae were collected in March 2014, the fourth and final instar larvae, pupae, adults were collected from June to July 2014. The second generation (non-wintering generation) appeared from July to September 2014. The first instar larvae were collected from July to August 2014. The second to fifth instar larvae and pupae appeared simultaneously in September 2014.

This species had a bivoltine life cycle with wintering and non-wintering generations (Fig. 6). In the wintering generation, clear development of instars was observed even in spring (from March to June). During non-wintering generation, the development increased from August to September (Fig. 6), when the daily mean temperature was more than 20 °C (Fig. 3). From October to February, larvae, pupae, and adults were not collected (Fig. 6); daily mean temperatures were below 20 °C in this period (Fig. 3).

All larvae and pupae were collected only from the surfaces of cobbles (Fig. 6), and no microhabitat shift was observed within their aquatic stage.

### ***Rhyacophila motakanta*** **(Site 2)**

The adults of this species were collected at Site 2 only in May 2014 (early summer season) (Table 1, Fig. 6). The first instar larvae appeared from September 2013

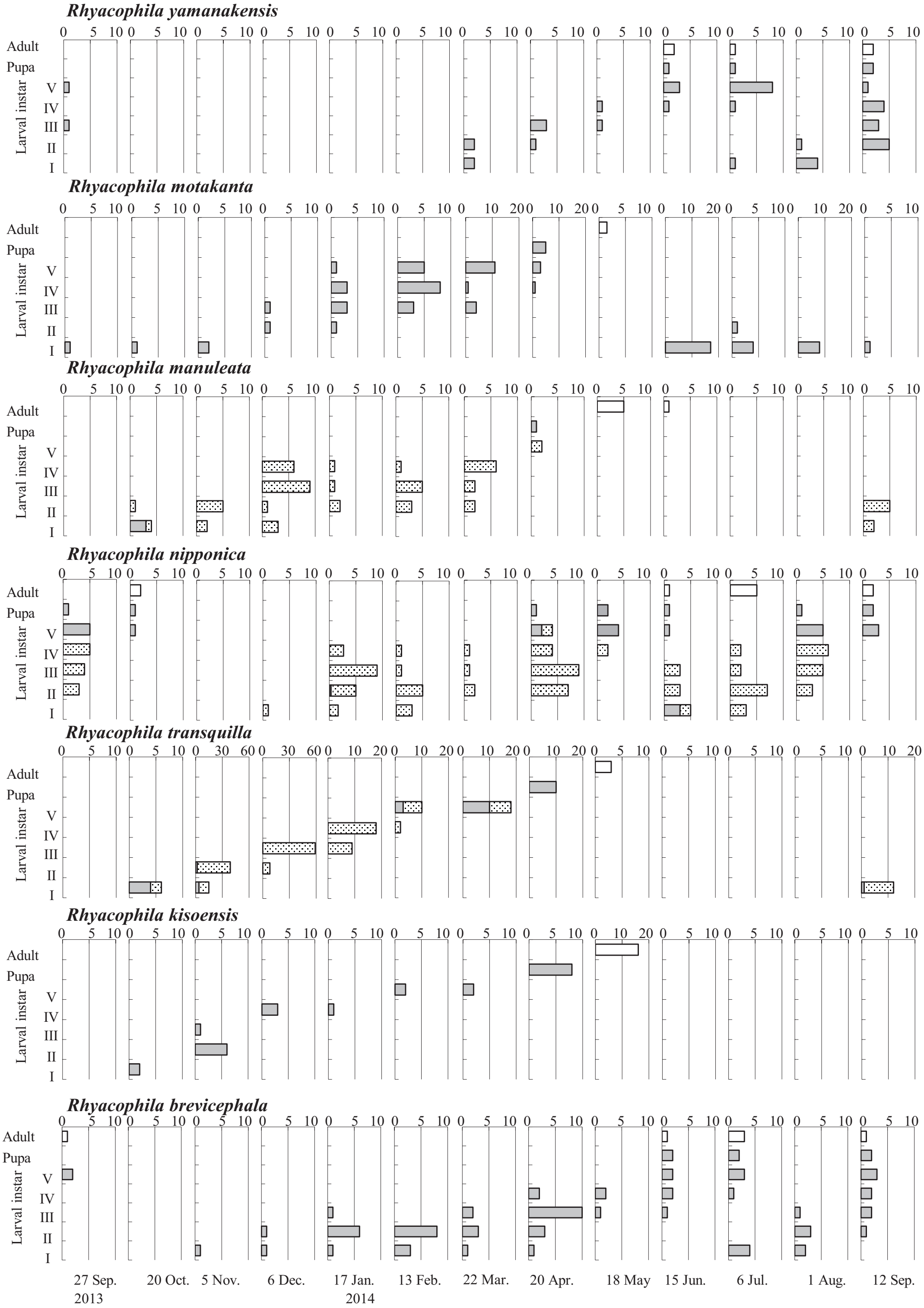

**Figure 6.** Life cycle patterns of seven *Rhyacophila* species at Site 2 showing the frequency of larval instars and pupae collected from each of the 30 quadrat samples (250 × 250 mm) from two types of streambeds; adults were collected by net sweepings and pan traps with black lights. In white rectangles (black border): adults collected by pan traps or by an entomological net. In grey rectangles: larvae and pupae collected from the surfaces of cobbles. In dotted rectangles: larvae and pupae collected from the interstices of gravels and sand.

to November 2014 and from June to September 2014, and were inferred to have been continually collected for seven months from June to November. The second and third instar larvae were collected from December 2013 to February 2014. The fourth instar larvae were appeared in March 2014, the fifth instar larvae appeared from January to April 2014; the pupae appeared in April 2014.

This species had a univoltine life cycle with early summer emergence (Fig. 6). They had the adult period of one month. The larval instar development was very slow, the development after the first instars were hardly observed from June to November (Fig. 6); even the daily mean temperature was over 20 °C during the period (Fig. 3).

All larvae and pupae were collected only from the surfaces of cobbles (Fig. 6), and no microhabitat shift was confirmed within their aquatic stage.

### *Rhyacophila manuleata* (Site 2)

This species appeared at the both sites, but numerous larvae were collected at Site 2 (Table 1). The adults of this species were collected from May to June 2014 (early summer season) (Fig. 6).

The first and second instar larvae appeared in October 2013 and September 2014. The second instar larvae were collected more often than the first instar larvae in November 2013. The third and fourth instar larvae appeared in December 2013, but the first and second instar larvae still remained. In January to March 2014, the second to fourth instar larvae were collected, the fifth instar larvae and pupae were collected only in April 2014. The peak of first instar larvae was recognized in October 2013, that of the second instar larvae in November 2013 and September 2014, whereas that of the third instar larvae in December 2013, and that of the fourth instar larvae in December 2013 and March 2014.

This species had a univoltine life cycle with early summer emergence season (Fig. 6). The clear development or progress of instars was observed even in winter (October to December) and spring season (March to April). But the development of the second to fourth instars was hardly observed from January to February (Fig. 6), when daily mean temperatures were less than 5 °C (Fig. 3).

Although most larva was collected from interstices of sand, some first instar larvae in October 2013 (and all pupae) were collected from surfaces of cobbles (Fig. 6). Since only the first instar larva was collected from both surfaces of cobbles and interstices of sand, I compared the wet weights of the first instar larvae between surfaces of cobbles and interstices of sand (Fig. 7). The larvae in interstices of sand were significantly heavier than those on surfaces of cobbles (Mann-Whitney U test: $P < 0.01$). All of the fifth instar larvae were collected from interstices of sand, but all pupae were collected from surfaces of cobbles (Fig. 6). This growth increment suggests that first instar larvae shifted microhabitat preferences, from surfaces of cobbles to interstices of sand, and the fifth instar larvae moved from interstices of sand to surfaces of cobbles to initiate pupation.

### *Rhyacophila nipponica* (Site 2)

This species appeared at both sites, but larvae were more abundant at Site 2 (Table 1). At Site 1, too few larvae were collected to clarify the life cycle. At Site 2, the adults of this species were collected from June to July and September, and two generations were recognized (Fig. 6).

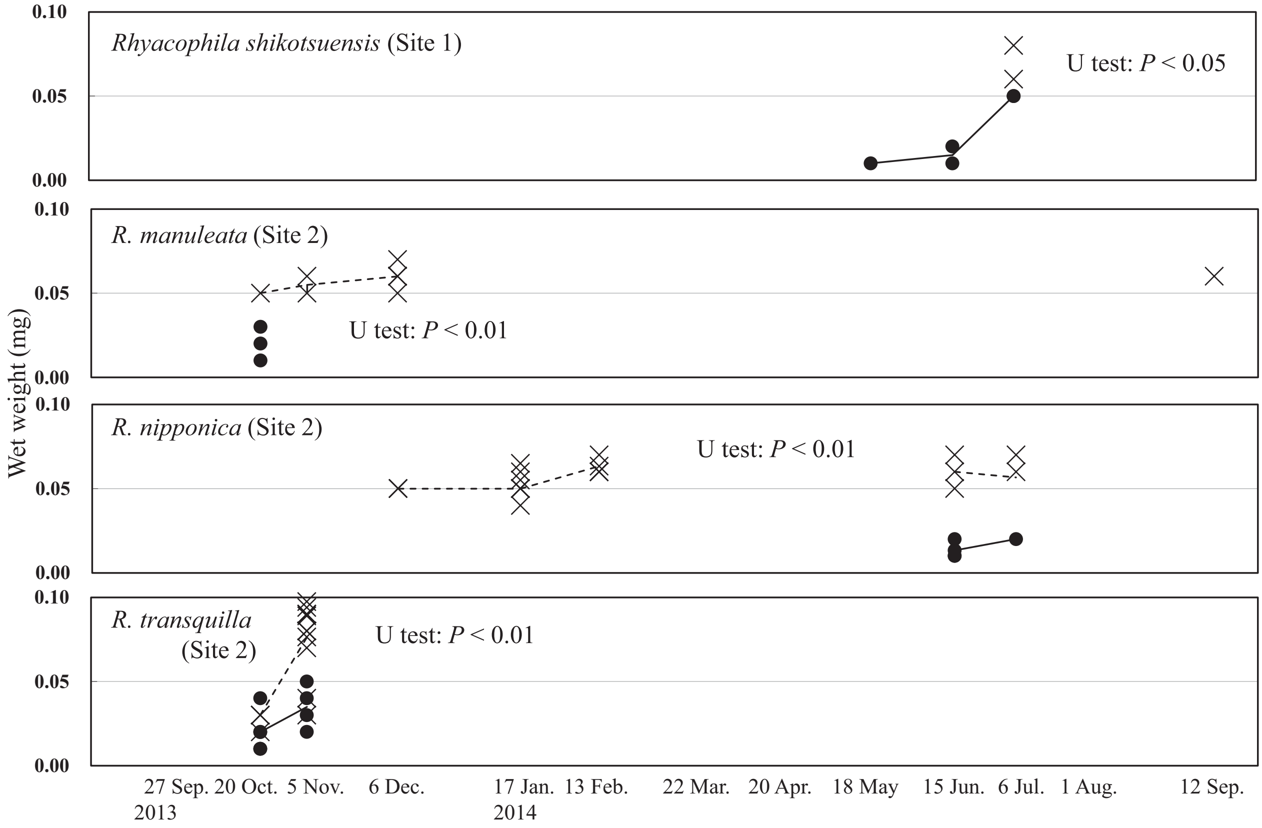

**Figure 7.** Seasonal changes of wet weight in the first instar larvae of *Rhyacophila* in the two microhabitats. Black circle: surfaces of exposed cobbles. X: interstices of gravel and sand.

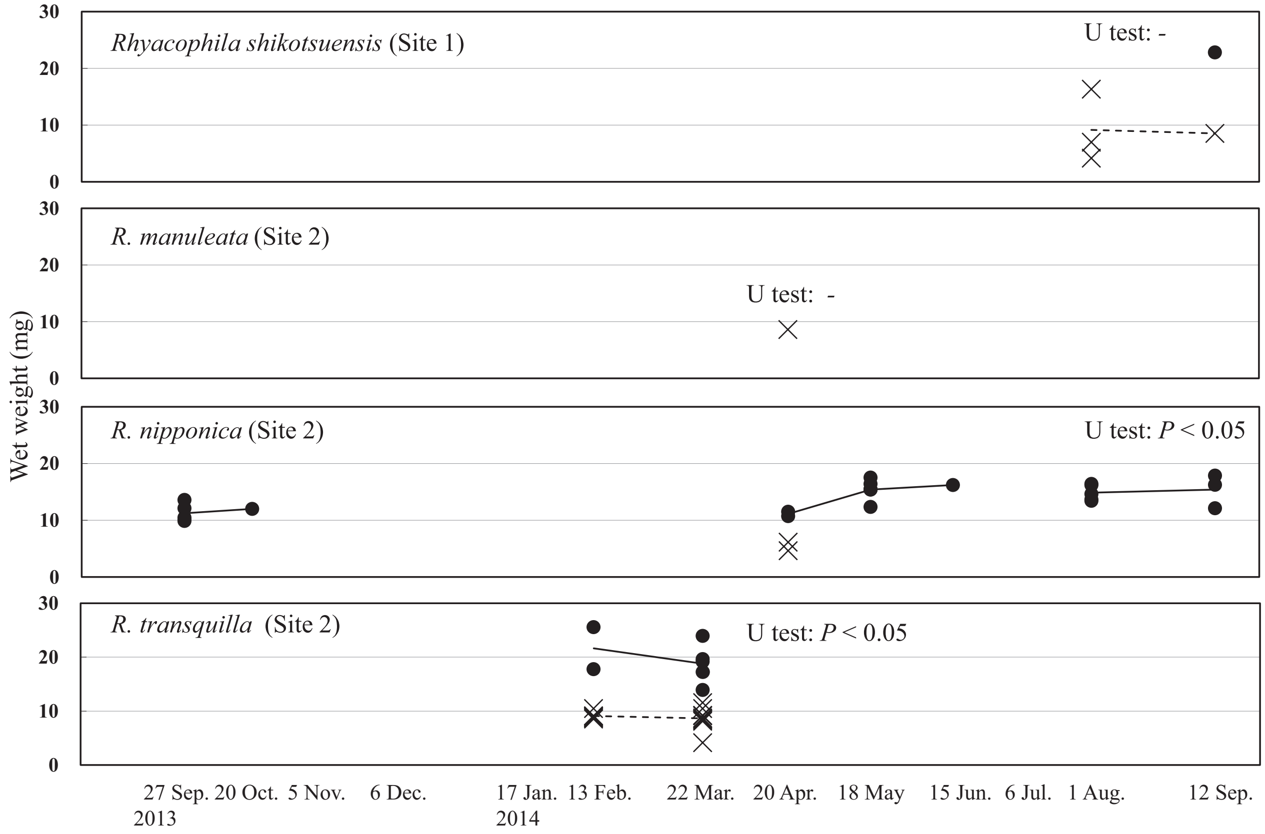

**Figure 8.** Seasonal changes of wet weight in the fifth instar larvae of *Rhyacophila* in the two microhabitats. Black circle: surfaces of exposed cobbles. X: interstices of gravel and sand.

The first generation spanned December 2013 to July 2014 (wintering generation), with first instar larvae observed in December 2014. The first to fourth instar larvae were collected January and February 2014, and no clear peak of the second to fourth instar larvae was detected in March 2014. The fifth instar larvae and pupae were collected from April to June 2014.

The second generation appeared to develop to maturity in the interval from June to September 2014 (non-wintering generation). First to third instar larvae were collected in June 2014; fourth instar larvae appeared concurrent with first to third instars while fifth instar larvae and pupae were collected in August and September 2014.

This species had a bivoltine life cycle, with a summer developing and an over-wintering generation (Fig. 6). In the over-wintering generation, instar growth and development were reduced from December 2013 to March 2014 (Fig. 6), when the daily mean temperatures were less than 8 °C (Fig. 3). In non-wintering generation, rapid development occurred from June to September 2014 (Fig. 6), when daily mean temperatures were more than 20 °C (Fig. 3).

All second to fourth instar larvae of both generations were collected from interstices of sand (Fig. 6). However, both the first instar and fifth instar larvae were collected from both surfaces of cobbles and interstices of sand. Pupae were collected only from surfaces of cobbles (Fig. 6). The first instar larvae were collected from surfaces of cobbles in June 2014 (the first month of non-wintering generation), but they were collected only from interstices of sand during other months. First instar larvae in interstices of sand were significantly heavier than larvae collected on surfaces of cobbles (Mann-Whitney U test: $P < 0.01$, Fig. 7). I infer that first instar larvae shift microhabitat preferences from the surfaces of cobbles to interstices of sand at some size threshold.

The fifth instar larvae of wintering generation appeared both at surfaces of cobbles and interstices of sand in April 2014, but were collected only from surfaces of cobbles in other months (Fig. 6). The fifth instar larvae on surfaces of cobbles were significantly heavier than those in interstices of sand (Mann-Whitney U test: $P < 0.05$, Fig. 9). We infer that fifth instar larvae shift habitat preferences from interstices of sand to surfaces of cobbles just before pupation.

### ***Rhyacophila transquilla*** **(Site 2)**

This species was collected in both sites, but larvae were collected in greater abundance at Site 2 (Table 1). At Site 1, only a few larvae were collected from October 2013 to February 2014.

At Site 2, the adults of this species were collected in May 2014 (Fig. 6). The first instar larvae appeared in October 2013 and September 2014; the second and third instar larvae appeared in December 2013. The third and fourth instar larvae were collected from January to February

**Figure 9.** Observation for spawning of *Rhyacophila nipponica* female from the side of the experimental tank. Arrows indicate females laying eggs on stone surfaces. The eleven species collected in this study also involved adult females laying eggs on stone surfaces.

2014, the fifth instar larvae were collected from February to March 2014. Pupae were collected in April 2014.

This species had a univoltine life cycle with the spring emergence season; clear development instar was observed from winter to spring (October to March) (Fig. 6). However, there was a period of four months from May to August without larvae and pupae, when the daily mean temperature was more than 15 °C (Fig. 3).

All second to fourth instar larvae were collected from interstices of sand (Fig. 6). However, first and fifth instar larvae were each collected from both interstices of sand and surfaces of cobbles. Pupae were collected only from surfaces of cobbles (Fig. 6). First instar larvae appeared from October to November 2013, and were collected from both surfaces of cobbles and interstices of sand. The wet weights of first instar were significantly heavier in interstices of sand than those on surfaces of cobbles (Mann-Whitney U test: $P < 0.01$, Fig. 7). We infer that first instar larvae shift their microhabitat preferences from the surfaces of cobbles to interstices of sand as a function of growth.

Fifth instar larvae appeared from February to March 2014, and were collected both from surfaces of cobbles and interstices of sand (Fig. 6). The wet weights of fifth instar larvae on surfaces of cobbles were significantly heavier than those captured from interstices of sand (Mann-Whitney U test: $P < 0.05$, Fig. 8). We infer that fifth instar larvae shifted their microhabitat preferences from interstices of sand to surfaces of cobbles before pupation.

### *Rhyacohphila kisoensis* (Site 2)

This species was collected at both sites, but larvae were collected in greater abundance at Site 2 (Table 1). Too few specimens were collected to assess the life cycle pattern of this species at Site 1.

The adults of this species were collected in May 2014 (Fig. 6). The first instar larvae appeared in October 2013, while the second and third instar larvae appeared in November 2013. The fourth instar larvae were collected from December 2013 to January 2014, the fifth instar larvae were collected from February to March 2014. Pupae were collected in April 2014.

This species had a univoltine life cycle, with a spring emergence season. Direct instar development was observed from winter to spring, similar to *R. transquilla* (October to March) (Fig. 6). However, there was a period of five months (from May to September) where we observed no larvae or pupae, and when the daily mean temperatures were more than 15 °C (Fig. 3).

All larvae and pupae were collected only from surfaces of cobbles (Fig. 6), and no microhabitat shift was confirmed during ontogeny in their aquatic stages.

### *Rhyacophila brevicephala* (Site 2)

This species appeared at both sites, but more numerous larvae were collected at Site 2 (Table 1). I can only show the life cycle of this species in Site 2. The adults of this species were collected from June to July and September 2014, and two generations were recognized (Fig. 6).

The first generation appeared from November 2013 to July 2014 (wintering generation), the second generation appeared during only three months from July to September 2014 (non-wintering generation). The first instar larvae were collected for six months from November 2013 to April 2014, and in July and August 2014. The second instar larvae were collected for five months from December 2013 to April 2014, and in September 2014. The third instar larvae appeared from January and September except February and July 2014, the fourth instar larvae appeared from April and September except August 2014. The fifth instar larvae and pupae were collected in June, July, and September 2014.

This species had a clear bivoltine life cycle with overwintering and non-overwintering generations (Fig. 6). In the overwintering generation, inconsistent development of larvae was observed from January to March, when the daily mean temperatures were less than 13 °C during this period (Fig. 3). In non-wintering generations, more consistent development was observed from July to September (Fig, 6), when the daily mean temperatures exceeded 20 °C (Fig. 3).

All larvae and pupae were collected only from surfaces of cobbles (Fig. 6), and no microhabitat shift was observed during their aquatic stage.

## The period without larvae and pupa

In the life cycles of four species, *R. yamanakensis*, *R. shikotsuensis*, *R. transquilla*, *R. kisoensis*, there was the period of two or three months in which there was no collection of larvae and pupae (Figs 5, 6). Egg-laying behavior of *R. yamanakensis*, *R. transquilla*, and *R. kisoensis* was observed in the field during May. Egg laying behavior of *R. shikotsuensis* was observed in November, using the rearing tank in my laboratory (Fig. 9, Taira unpublished). Females of all four species were observed laying eggs on stone surfaces. Therefore, it seems that these four species were dormant in the egg stage during the period without larvae and pupae collection.

# Discussion

## Life cycles of Japanese *Rhyacophila* species

In Europe and North America, many *Rhyacophila* species, e.g., *R. dorsalis* (Curtis, 1834), *R. minora* Banks, 1924, *R. munda* McLachlan, 1862, have a univoltine life cycle (e.g., Elliott 1968; Singh et al. 1984). Some species, e.g., *Rhyacophila vao* Milne, 1936 *R. brunnea* Banks, 1911, and *R. tristis* Pictet, 1834, have a semi-voltine life cycle and completes a life cycle over the course of two years (Dixon and Wrona 1992; Dobrin and Giberson 2003). Therefore, the life cycles of *Rhyaacophila* species are diverse.

In the present study, I surveyed the life cycles of eleven Japanese *Rhyacophila* species. These data suggest that eight of these species have univoltine life cycles, and three species bivoltine. For those eight species with univoltine life cycles, seven species, (*R. lezeyi*, *R. orthacantha*, *R. clemens*, *R. motakanta*, *R. manuleata*, *R. transquilla*, *R. kisoensis*) mostly emerged from spring to early summer. One species, *R. shikotsuensis*, emerged in autumn. Among those seven species emerged from spring to early summer, the timing of adult emergences differed.

In addition to the eleven species in this study, *R. nigrocephala* and *R. kuramana* Tsuda, 1942 are known to have bivoltine life cycles (Taira et al. 2013; Taira 2018). Both species were distributed also in the Kinokawa River. *Rhyacophila hokkaidensis* Iwata 1927, which inhabit in the Izari Stream of Hokkaido (42.857°N, 141.411°E), has semi-voltine life cycle and completes its life cycle for two years (Ito 1999).

Geographical variation in the life cycle of some Japanese *Rhyacophila* species has been reported. *Rhyacophila nipponica* has a bivoltine life cycle in this study, but in the Izari Stream of Hokkaido (42.857°N, 141.411°E), this species appears to have a univoltine life cycle, inferred from its emergence season (Kuhara 2011). In a huge spring fed stream, the Kakida River in central Honshu (Shizuoka Prefecture, 35.101°N, 138.901°E), where there is a stable temperature of 15 °C throughout the year, Malaise trapping of adults (Nozaki and Tanida 2007) suggests that *R. nipponica* has a bivoltine life cycle. *Rhyacophila lezeyi* had a univoltine life cycle in the present study. However, where hot springs influence stream temperatures to remain higher than 5 °C even in wintertime, (Aoya and Tanida 2023) report this species to exhibit a bivoltine life cycle with long adult flight periods.

## The period without collecting larvae or pupae in the streams

In the present study, *Rhyacophila shikotsuensis*, *R. manuleata*, *R. transquilla*, *R. kisoensis*, and *R. yamanakensis*, had fairly long periods without collecting larvae or pupae (hereafter referred to as “blank periods”). Although I extensively sampled both the surfaces of cobbles but also interstices of gravel and sand, no larvae or pupae were collected during some periods. However, neither did I collect eggs during these periods at the site. I observed the oviposition of those four species in the aquariums before the beginnings of the blank periods (Fig. 9, Taira unpublished). The oviposition of these species occurred before the blank periods, and the eggs seem to not immediately initiate development. Some records of Trichoptera report egg diapause in blank periods (e.g., Anderson and Bourne 1974; Bowlesm and Allan 1992). As an example of a longer period of dormancy, *Agapetus bifidus* Denning, 1949 eggs in Oregon were recorded as dormant for 8 to 10 months (Mackay and Wiggins 1979). Therefore, egg diapause is the most likely explanation for these observations.

The blank periods of three species, *R. manuleata*, *R. transquilla*, and *R. kisoensis*, were during warm water temperature regimes, from June to August (17–26 °C), but the blank period of *R. shikotsuensis* occurred under colder water temperature from December to April (2–9 °C). High (more than 17 °C) and low (less than 9 °C) temperature themselves seem to be a major reason to prevent the developments of eggs.

*Rhyacophila yamanakensis* also had blank periods and an egg diapause seemed to occur from October 2013 to February 2014. However, unlike the other four species, the water temperature during the blank periods for this species varied widely, from 1 to 20 °C. Therefore, it is necessary to check the spawning period and confirm whether egg diapause has occurred.

## Periods with minimal larval development

Some species had a period with no obvious larval growth or development. For *R. lezeyi* this period was during colder water temperatures, from January to February. Aoya and Tanida (2023) estimated the developmental zero temperature of the pupae of this species to be 6.8 °C under a rearing experiment. Although the developmental zero temperature of the larvae is unknown, their growth seemed to be halted due to the colder water temperature during the period from January to February (1.4–6.8 °C)

For other species (*R. orthacanta*, *R. motakanta*, *R. clemens*), periods of minimal larval development were observed in two seasons of colder water temperatures from January to February (1.4–6.8 °C) and warmer water temperatures from June to November (15.0–26.2 °C). I suggest that the colder water temperatures in January and February may be below the developmental zero temperatures of these three species. Additionally, higher temperatures during June to November may also inhibit or prevent larval growth and development at these sites.

## Species richness of *Rhyacophila* in the upper reaches of the Kinokawa River

In this study, I found eleven *Rhyacophila* species inhabiting the upper reaches of the Kinokawa River. One possible explanation why so many species are able to coexist is the differences in the water temperature regime between the two sites. Wintertime water temperatures were similar at the two sites, but summer water temperatures in summer diverged by about 5 °C. The differences in summer temperatures between the two sites enhanced the species richness of principally cold-adapted *Rhyacophila* species, by catering to particular preferences of the larvae of different species.

The second reason is the differences of microhabitats preferences between species. The larvae of many species live on the surfaces of cobbles, but the larvae of four species, *R. shikotsuensis*, *R. manuleata*, *R. nipponica*, and *R. transquilla*, live in the interstices of gravel and sand. Although not shown in this study, *R. kuramana* also occur at these study sites (Taira et al. 2013), inhabiting rock surfaces in spring seepage zones. Therefore, habitat diversity, and a diversity of preferences among species, promotes the coexistence of many species in the Kinokawa River system.

A third factor may be seasonal partitioning of the emergence periods between the seven species. Emergence periods appear to be staggered such that species minimize co-occurrence during adult stages, but the mechanisms governing such patterns are unknown. If there is a cost to simultaneous emergence, the distribution of adult emergence in this system is apparently timed to enhance the species richness of *Rhyacophila*.

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