Limnology (2007) 8:85–93
DOI 10.1007/s10201-007-0203-7

RESEARCH PAPER

Ken Miyairi · Koji Tojo

Biology of the mayfly *Bleptus fasciatus* Eaton (Insecta: Ephemeroptera, Heptageniidae), with special reference to the distribution, habitat environment, life cycle, and nuptial behavior

Received: February 27, 2006 / Accepted: January 28, 2007 / Published online: July 7, 2007

Abstract As the habitats of the mayfly *Bleptus fasciatus* (Ephemeroptera, Heptageniidae) are unique and specialized, information on the distribution and biology of the mayfly is very poor. In this article, the biology of the mayfly is described, with special reference to the distribution and life cycle: the voltinism, emergence and reproductive season, nuptial behavior (i.e., swarming), egg period, and the early stage of postembryogenesis. The life cycle of the mayfly was judged as semivoltine, in contrast to the previous interpretation. Also, the unique nuptial behavior or the swarming of the mayfly is revealed.

Key words Mayfly · Life cycle · Voltinism · Swarming · Embryogenesis

Introduction

The genus *Bleptus* is the smallest group of mayflies, with only one species described, *Bleptus fasciatus* Eaton, 1885 (Eaton 1885). The mayfly has been thought formerly to be endemic to Japan (Gose 1985), but it has been recently reported from the Korean Peninsula (Bae et al. 1994; cf. Ishiwata 2001; and our field research). Ephemeroptera is regarded as one of the representatives closest to early pterygote ancestors, and moreover, *B. fasciatus* has many ancestral characteristics and is regarded as the basal position in the mayfly phylogeny. *B. fasciatus* is a "key species" in understanding the subsequent evolutionary paths and the diversity of winged insects, the Pterygota.

Information on aquatic insects or benthic fauna of Japanese rivers has been rapidly accumulating as a consequence of the national project "National Censuses on River Environments" (hereafter abbreviated NCRE), conducted by the Japanese Ministry of Land Infrastructure and Transport and some self-governing bodies, which started in 1990. However, information on the distribution and biology of this mayfly is still very poor, because the habitats of the mayfly are very unusual, for example, seepage zones, petrimadicolous or hygropetric habitats of headwater areas, or at the minute flow and splash zones of small waterfalls. Headwaters are unique components of catchments as they usually support a taxonomically and ecologically unique fauna, but the habitat borders are generally narrow and have a fluctuating course. Headwaters have received little attention from ecologists, and their benthic fauna, including insects, are poorly studied.

On the other hand, the habitats of this mayfly are in mountainous areas, especially at the kind of place where cliff erosion often occurs. The forest and mountain roads in these environments are undergoing legal surface construction (Tojo and Miyairi 2005; Miyairi and Tojo 2007; cf. Tojo 2005). So, *B. fasciatus* is often listed as a threatened species by regional or local governments (e.g., at Gunma, Nagano, and Okayama Prefectures). For this reason, basic and detailed research of the mayfly should be important.

Accordingly, we have started research on the distribution or biogeography, and basic biological research into *B. fasciatus*. In the first part of our studies, we outline the distribution and life cycle of the mayfly, with special reference to voltinism, emergence, and reproductive season, nuptial behavior (swarming), egg period, and the early stage of postembryogenesis. This article is based on field research throughout a year and observation in the laboratory.

K. Miyairi
Department of Environmental System Science, Graduate School of Science and Technology, Shinshu University, Nagano, Japan

K. Tojo (✉)
Department of Biology, Faculty of Science, Shinshu University, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
Tel. +81-263-37-3341; Fax +81-263-37-2560
e-mail address: ktojo@shinshu-u.ac.jp

K. Tojo
Institute of Mountain Science, Shinshu University, Nagano, Japan

Materials and methods

Geographic distribution

We gathered data on distribution of the mayfly *B. fasciatus* based on our own field research and on our surveys of published literature and records.

Life cycle and nuptial behavior

Study site. This study was conducted in the headwater area of a small brook, Fujii-sawa, in Matsumoto (Nagano Prefecture, Central Japan: 36°14′ N, 138°01′ E, 840 m altitude; Fig. 1). This site is near the type locality in Yabuhara, Kiso (Nagano Prefecture) at a distance of about 40 km. The Fujii-sawa is a small tributary of the Shinano (Chikuma) River system (a first-level tributary), in high seepage environments, and includes splash zones of small waterfalls. *B. fasciatus* inhabit this small area at a high density.

Sampling and observation. Mayfly nymphs were collected biweekly by random sampling throughout a year (13 months), from October 8, 2004 to October 26, 2005 at the study site mentioned above. Samples at each of the field research sites were randomly taken during a specific time (30 min) with soft forceps in the small headwater area (about 200 m^2). Only the individuals that were clearly distinguished as the species *B. fasciatus* were collected (only specimens larger than 2.5 mm body length or 0.7 mm head width were selected to be examined). Their body size, i.e., body length (from the anterior margin of the head to the posterior margin of the tenth abdominal tergite: excluding antennae, cerci, and the caudal filament) and head width, were measured to the nearest 0.1 mm using a binocular microscope with an objective micrometer.

The last-instar nymph specimens were measured discriminately between males and females, as the body size of females was clearly larger than that of males ($P < 0.001$, $n = 240$). Old instar nymphs (not only the last-instar nymphs) probably also retain the sex-based difference in body size; however, we could not perfectly distinguish the sex by external observation. Thus, the nymphs except those in the last-instar stage were treated without differentiating sex.

Subimagos and imagos were collected with collecting nets: sweeping the leaves of trees or plants, or with a light trap using a 500 W mercury lamp near the sampling site for nymphs. This phase occurred from May 9, 2005, when the last-instar nymphs were observed, to August 10, 2005.

We observed the reproductive behavior, with reference to the nuptial flying dance or the swarming in the field at

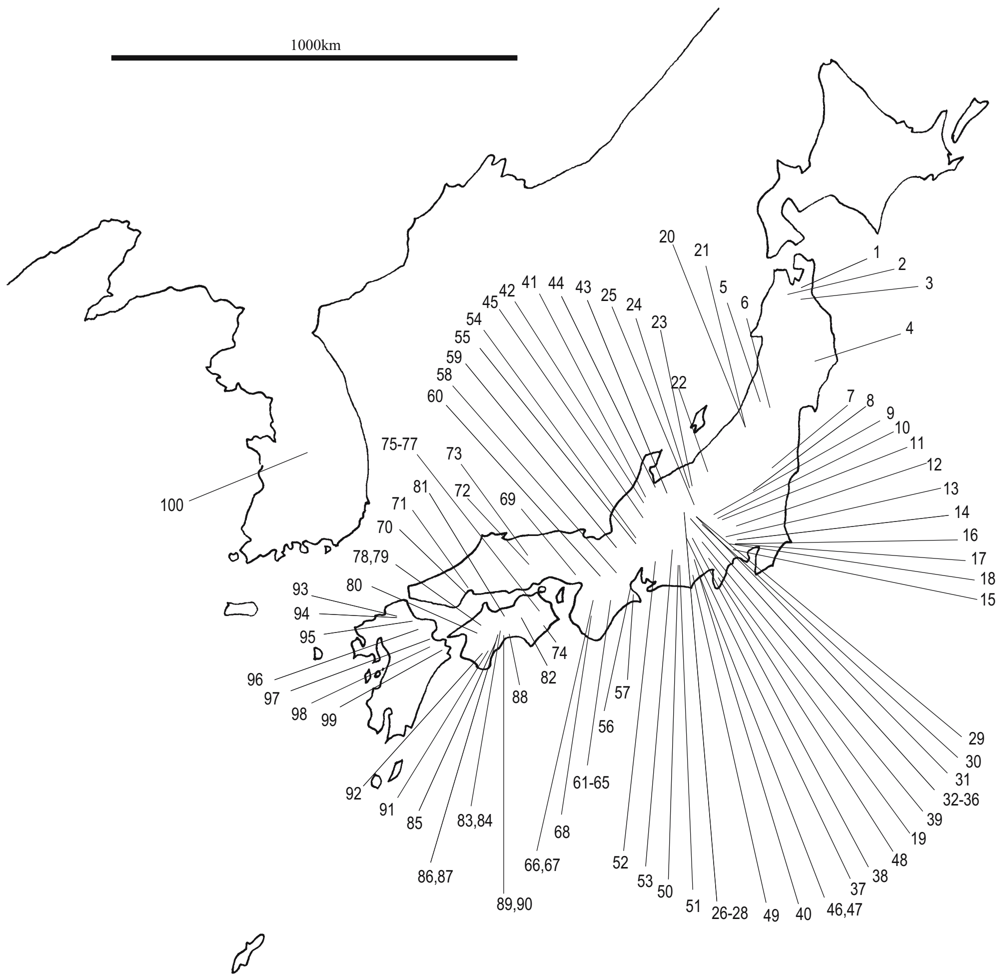

Fig. 1. Map showing the distribution of *Bleptus fasciatus*. The *number* of each locality corresponds to that in Appendix 1. Site number *38* is the type locality, Yabuhara, Kiso, Nagano Prefecture (cf. Miyairi and Tojo 2007); site *29* is our main study site, Fujii-sawa, Matsumoto, Nagano Prefecture

the emergence season (from early June to late July). Mated, mature females of the mayfly were collected at the study site, and eggs were obtained in the laboratory. The eggs were incubated in DW (distilled water) at 21° ± 1°C (the temperature was set with reference to the water temperature in the field) during the egg developmental period. After hatching, nymphs of the early instar were also incubated, were observed during early postembryogenesis, and were studied to understand the life cycle.

Results

Geographic distribution, habitat, and environments

Forty-four new localities were listed found through our field research. The new geographic distribution of the mayfly *B. fasciatus* is shown in Fig. 1, including the localities data from some published literature (e.g., Ishiwata 1997a,b, 2002). As shown in Fig. 1, *B. fasciatus* is endemic to East Asia (mainly distributed in Japan: Honshu, Shikoku, and the Kyushu Islands). They are not recorded from Hokkaido Island or from the southern section of Kyushu Island.

The habitat environments observed so far in each case are in the seepage zone, the petrimadicolous or hygropetric zone of headwater areas, or at the minute flow and splash zones of small waterfalls (Fig. 2A). This species is found on the wet surface of rocks or stones at the habitats already mentioned (Fig. 2B).

Fig. 2. Study site environment at Fujii-sawa, Matsumoto, Nagano Prefecture (**A**). The study site is in a high seepage environment, and includes the splash zone of small waterfalls. *Bleptus fasciatus* inhabits this small area at a high density: nymphs just before emergence (**B**). Female subimago just after emergence (**C**). (Photographs by M. Uenishi)

Life cycle and nuptial behavior

Semimonthly field research for understanding the basic biology (e.g., life cycle, reproductive behavior) was executed throughout a year at a small brook, Fujii-sawa, in Matsumoto. Body size (body length and head width) of each collected nymph was measured. A strong correlation between the body length and head width was recognized (coefficient correlation $R^2 = 0.8473$; Fig. 3). In this article, the data of head width was used, as the head capsule is chitinized to become very hard and stable (e.g., expansion and contraction under various conditions).

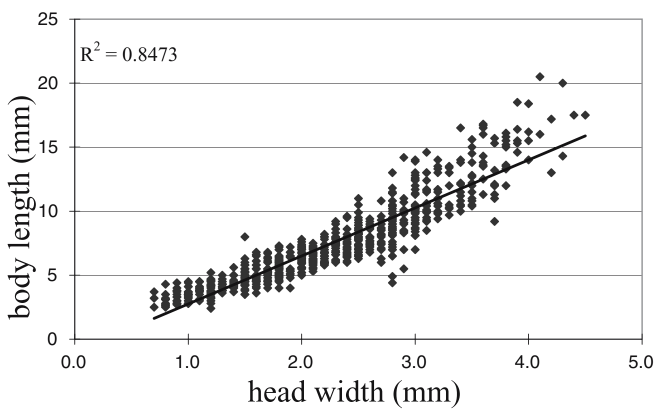

Fig. 3. Body length and head width relationship of *Bleptus fasciatus* nymphs ($n = 738$). A strong correlation was recognized

Figure 4 shows the frequency distribution of head widths of the *B. fasciatus* nymphs collected on each sampling occasion at the study site. The various sized nymphs that occurred were collected throughout the duration of the field research from October 2004 to October 2005. Emergence to the adult stage continued from early June (June 9 was the earliest) to late July (July 28 was the latest). The dates when molt to imaginal stage, i.e., emergence, was observed, are indicated (by arrow) in Fig. 4. The emergence season is relatively short, once a year. The life of the emerged mayfly observed in the laboratory was 3 to 6 days including about 1.5 days at the subimaginal stage. The sample included 26 males and 6 females ($n = 32$), and no difference between males and females was noted. Therefore, the imagos could be observed perhaps up until early August (3–6 days later from the last emergence). The short imaginal life of *B. fasciatus* is a general characteristic of mayflies (Peters and Campbell 1991). From the foregoing field research and laboratory data, the reproductive period of the mayfly is thought to be from early June to early August.

Emergence occurs during daytime (in many cases in the afternoon, but rarely during ante meridiem), and the subimagos after emerging rest on leaves motionless for 1 or

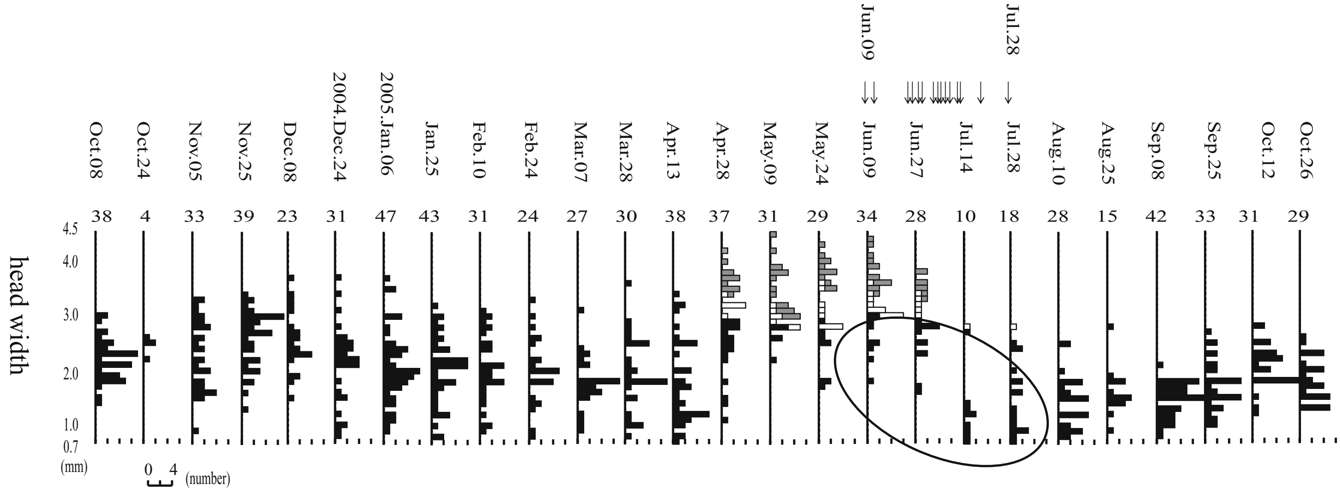

Fig. 4. Bimonthly recordings of *Bleptus fasciatus* body size distribution (head width) from October 8, 2004, to October 26, 2005, at the Fujiisawa study site (Matsumoto, Nagano Prefecture, central Japan). Only the last-instar nymphs were treated discriminately between males (*gray*) and females (*no color*). Other nymphs (*black*) not in the last-instar stage were treated without differentiating sex. *Arrows* represent the dates when the emergence to imaginal stage was observed. (Refer to text for explanation of *circled* portion)

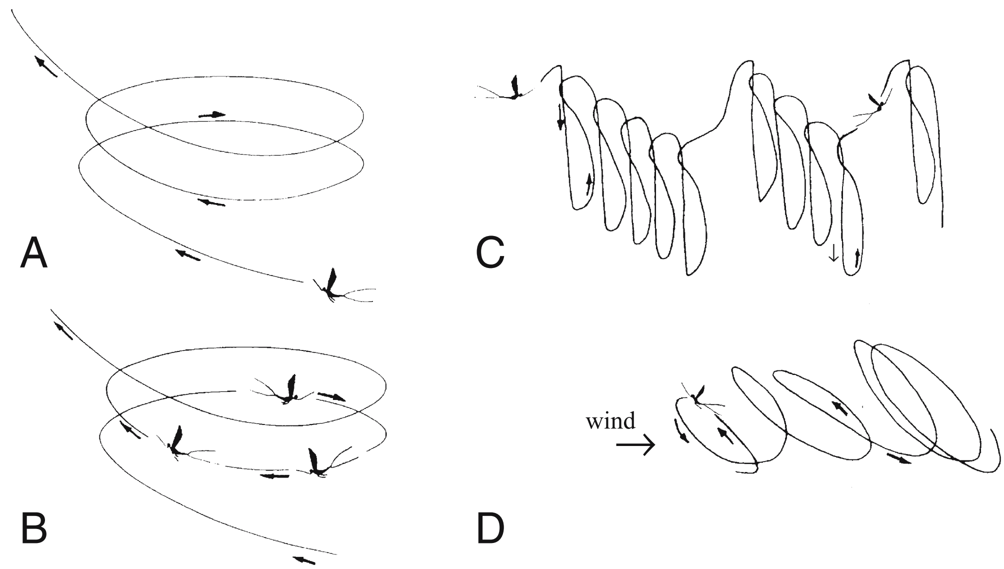

Fig. 5. Flight trajectory of the male(s) of *Bleptus fasciatus* in the nuptial dance (**A**, **B**; refer to text for explanation). Typical nuptial dances of mayflies (e.g., *Ephemera vulgata* of a ephemerid mayfly) in still air (**C**) and in wind (**D**) (after Brodskiy 1973; see text). The *arrow* indicates wind direction

1.5 days in the shade under or on the leaves of broadleaf trees, around the nymphal habitat. The subimagos molted to the imaginal stage at almost the same location. Imagos were also observed in the same resting place as that of subimagos, during daytime. When it was evening (in almost all cases at 1530–1730 in the emergence season), males flew for pairing, i.e., "swarming," at just several meters above the nymphal habitat. In mayflies, the swarming for pairing itself has been regarded as the normal conduct (Brodskiy 1973). In this species, however, males fly up and begin revolutions as if in drawn circles on a plane at a regular height (Fig. 5A). Their nuptial flights are very fast. The swarming trajectory was circular, horizontal to the ground (the circle was approximately 0.5–2 m in diameter), and the swarming pattern of this species was different from any other type of mayfly known so far (cf. Fig. 5A vs. Fig. 5C,D). In addition, it was often observed that two or three males flew in the same circular trajectory.

Eggs were obtained in the laboratory from mated and mature females, although the circumstances of egg laying in the field were not observed. The eggs were incubated in water at $21° \pm 1°C$. The egg developmental (embryogenesis) period of the mayfly ranges from 21 to 22 days at a steady temperature. In our observations following hatching, the first nymphal stage was about 4 days, the second stage was about 5 days, and the third stage was about 6 days (Table 1). For the subsequent stages, sufficient data were not obtained, as incubation is very difficult.

Table 1. Timeline of the embryogenesis and early postembryonic stages (1st- to 3rd-instar nymphal stages) of *Bleptus fasciatus* at 21° ± 1°C incubation

Stage	Period	Head width
Egg (embryo)	~22 days (21–22 days)	<0.1 mm[a]
1st-instar nymph	~4 days (4–6 days)	~0.1 mm
2nd-instar nymph	~5 days (4–6 days)	~0.1 mm
3rd-instar nymph	~6 days	~0.1–0.2 mm

[a] Head width of embryo (just before stage of hatching)

Discussion

Geographic distribution, habitat, and environment

Although up-to date knowledge regarding the distribution of the mayfly *B. fasciatus* was gathered as shown in Fig. 1, information on the mayfly is still insufficient. As a consequence of the NCRE project started in 1990, information on the aquatic insect fauna of Japanese rivers has been rapidly accumulating. However, information about the mayfly *B. fasciatus* has not accumulated quite so readily because of the unique and specialized habitats of the mayfly (see Fig. 2). The mayfly group containing the genus *Bleptus* is endemic to East Asia, and only one species in the genus, *B. facsciatus*, is key to understanding the evolutionary paths and the diversity of pterygote insects. Therefore, further research on *B. fasciatus* would be of great benefit.

Furthermore, many habitats of the mayfly are in mountainous areas, especially, at the shallow and slowly flowing brooks or in marginal regions of rapid streams (Uéno 1931), and at the kind of place where cliff erosion often occurs. The forest and mountain roads in these environments often require manmade reinforcement of surface construction. So, the mayfly is often listed as a threatened species by regional or local governments. For this reason, basic and detailed research of the mayfly is desirable and should be continued in the future.

The life cycle

The emergence of the mayfly *B. fasciatus* was observed at the study site for only a short period, from early June to late July (see Fig. 4). In addition to the laboratory data on subimago and imago incubation, it is considered that the reproductive season of this species is a short one in early summer, i.e., early June to early August. Therefore, it has been thought that the mayfly is univoltine, as this mayfly is seen in just one short period per year (Gose 1985). Clifford (1982) suggests that life cycles of the majority of Heptageniidae are univoltine.

In this study, on the other hand, the mayfly nymphs of various sizes occurred throughout the duration of sampling from October 2004 to October 2005 (see Fig. 4). Moreover, in the reproductive season (from early June to early August), many small nymphs were also observed (for example, the nymphs as shown in the circled area in Fig. 4). These small nymphs were probably not able to emerge in the summer of 2005, i.e., two nymphal cohorts coexist during the emergence season: (1) the large nymphs just before emergence, and (2) the small nymphs that would be able to emerge next summer. In addition, our observations of the embryogenesis and postembryogenesis in the laboratory revealed (see Table 1) that the coexistent small nymphs were not individuals that were hatched at the early summer of the same year, 2005, but were hatched approximate 1 year prior, because the egg period was about 22 days and, based on the early postembryogenesis data (see Table 1), the half-month was necessary for the hatched nymphs to reach the 4th-instar stage (head width is at the longest, 0.2 mm, at the 4th nymphal stage). So, the mayflies could not grow enough in 1 month or even 1.5 months to reach the size of the coexistent small nymphs identified as the species *B. fasciatus* in the field (body length, about 2.5 mm; head width, about 0.7 mm, as shown in Fig. 4). We consider that the nymphs as shown in the circle of Fig. 4 probably were the individuals originating from eggs laid in the previous year. From the seasonal trend of the size distributions in Fig. 4, it appears that contrary to the previous interpretation (e.g., Gose 1985), the life cycles of the mayfly are semivoltine.

On the basis of the foregoing information, life history of the mayfly *B. fasciatus* probably will be as follows. Emergence and reproductive season is early summer; early June to early August. Nymphs hatch from late June to late August, and probably grow slowly from fall extending through the early winter (but as yet they are rather small, and have not appeared in Fig. 4). After winter at the beginning of the next year, they grow to reach a 0.7 mm head width. However, their growth is still slow. After the second spring, their growth rate accelerates a little, and their emergence and reproductive season is during the second summer.

This investigation was one undertaken at the Fujii-sawa study site (Matsumoto, Nagano Prefecture, Japan), but a similar pattern of life cycle was also recognized in other localities, although in those cases the research was less rigorous. For example, in some localities of the Kansai district, small nymphs were also observed during the emergence season, as at our study site. The mayfly requires 2 years until it reaches the imaginal stage. The life cycle of the mayfly is a long one, needing as long as 2 years to complete. Yet, perhaps life cycle length depends on low-temperature water flow. For example, at this study site, water temperature decreases to levels close to 0°C around wintertime (from late November to early March). In contrast, during summer the water temperature does not rise very high, perhaps reaching only 15°C for a short time. Certainly, some authors suggest that water temperature is a major factor determining egg development and nymphal growth in their studies and reviews of the ephemeropteran life history (Elliott 1967; Sowa 1975; Humpesch 1979; Clifford 1982; Brittain 1982, 1990).

The nuptial behavior

Among mayflies, generally, nuptial behavior or dance for pairing, i.e., the swarming for pairing itself, has been regarded as the usual conduct (Brodskiy 1973; Savolainen 1978). However, the swarming of the mayfly *B. fasciatus* is unique even among all ephemeropterans. The nuptial behavior of the male mayfly is shown in Fig. 5A. The swarming trajectory of this mayfly is circular, horizontal to the ground, in windy conditions and also in nonwindy conditions. In addition, it was often observed that two or three males swarmed in the same circular trajectory (Fig. 5B). In many cases, the swarming mayflies were observed to selectively swarm in the bright zone where the evening sun was shining above the nymphal habitat.

The swarming of mayflies has been described frequently since the 17th century (Swammerdam 1675). According to a thorough review of mayfly swarming (Brodskiy 1973), it can be classified into four types of swarming behaviors as follows. (1) The most general type is characterized by the following five characters as (a) to (e): (a) the swarm consists of males; (b) the swarm is located above a marker; (c) the males in the swarm demonstrate nuptial dances, most often a vertical dance, consisting of active and passive components (Fig. 5C); (d) the female flies through the swarm and is captured by a male; and (e) the male detects the female visually. This swarming pattern is recognized in many mayfly groups, including Ametropodidae, Baetidae, Baetiscidae, Ephemelleridae, Ephemeridae, Heptageniidae (part of the genera), Isonychiidae, Leptophlebiidae, Metretopodidae, Potamanthidae, and Siphlonuridae. (2) The second type of swarming occurs in some species belonging to the genera *Heptagenia* and *Stenonema* (Heptageniidae). Unlike the first type of swarming, the nuptial dance of the male of this group assumes the form of hovering. (3) Here, swarming differs sharply from the first and second types. The nuptial dance of the male is represented by rapid horizontal flights parallel to the surface of the water (Oligoneuriidae, Palingeniidae, and Polymitarcyidae). (4) The nuptial dance of the male is analogous to that of swarming in the first type; both males and females may participate in swarming (occurs in species of Caenidae and possibly of Trichorythidae).

Although the swarming of *B. fasciatus* is an unique type not previously observed, it is considered that the swarming of this mayfly is a largely modified case of type 1. Almost all type 1 male mayflies take off almost vertically (sharply at 90°), and their nuptial dance sequence of active (upward flying) and passive (gliding falling) components is repeated several times, during which the male rises somewhat higher, at the same time shifting horizontally (Fig. 5C). However, the male may sometimes be inclined to fly partly horizontally at angles of up to 75°–80°, especially under windy conditions (Fig. 5D; Brodskiy 1973). Even in comparison with this, the trajectory of this mayfly *B. fasciatus* is an exceptional case, i.e., completely horizontal (0°; see Fig. 5A,B). This peculiarity is, perhaps, related to the unique environmental habitat. The unique swarming of this mayfly may have been acquired or modified to adapt to the extremely narrow space for pairing at the headwater area.

Acknowledgments We acknowledge the support of Drs. R. Kuranishi (Natural history Museum and Institute, Chiba) and T. Nozaki (Kanagawa Environmental Research Center), Y. Takemon (Kyoto University), Mr/s I. Mori (Okayama Prefectural Nature Conservation Center), I. Yoshitaka (Kanzei High school), M. Uenishi (Ryukoku Univiversity), T. Torii, T. Kyuka and G. Yoshinari (Metocean Environment Inc.), K. Nio (Kochi City), N. Kawase (Minakuchi Nature Center), N. Taniguchi (Kagawa University) for their cooperation in collecting materials. We are also indebted to Messrs. T. Tanizawa, K. Suzuki, K. Sekiné, K. Oka and Y. Morii (Shinshu University) for their cooperation in field research at the study site. This study was supported by the grants for River Environment Fund (17-1215-19) of Foundation of River & Watershed Environment Management, and for Interdisciplinary Research Institute of Environmental Sciences, and by the Grant-in Aid for Scientific Research from the Japanese Society for the Promotion of Science (JSPS: no. 16510180) to R. Kuranishi.

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Appendix

Detailed locality information of known *Bleptus fasciatus* Eaton habitats shown in Fig. 1

No. in Fig. 1	Locality				Collected date	Stage of collected samples[a]	Collecting method[b]	Reference
	Site	Prefecture	Latitude	Longitude				
Japan								
1	Aomori (Otsubo-gawa)	Aomori	40°46′ N	141°01′ E	Aug. 5, 2000	Ns	S	Present study (collected by Yoshinari)
2	Kuroishi (Aseishi-dam)	Aomori	n	n	1999	Ns	SN	NCRE[c]
3	Towada (Oirase-gawa)	Aomori	n	n	June 9, 2001	Ns	S	Present study (collected by Yoshinari)
4	Hanamaki (Tase-dam)	Iwate	n	n	2001	n	SN	NCRE[c]
5	Tsuruoka (Gassan-dam)	Yamagata	n	n	2003	Ns	SN	NCRE[c]
6	Nishikawa (Sagae-dam)	Yamagata	n	n	2000	n	SN	NCRE[c]
7	Nishigo (Yanta-gawa)	Fukushima	37°09′ N	141°01′ E	Sept. 23, 2003	Ns	S	Present study (collected by Yoshinari)
8	Katashina (Tokura-dam)	Gunma	n	n	n	Ns	n	–[d]
9	Shimonita (Nanmoku-gawa)	Gunma	36°01′ N	138°43′ E	Mar. 29, 2005	Ns	S	Present study
10	Ueno (Nakakoshi-sawa)	Gunma	36°05′ N	138°44′ E	Mar. 29, 2005	Ns	S	Present study
11	Kamiru (Mamono-sawa)	Gunma	36°04′ N	138°49′ E	Mar. 29, 2005	Ns	S	Present study
12	Chichibu	Saitama	n	n	n	n	N, SN, LT	Saitama Prefecture web site
13	Akiruno (Aki-kawa)	Tokyo	n	n	June 22, 1991	Ns	n	Ishiwata (1997b)
14	Hachioji (Hikage-sawa)	Tokyo	35°38′ N	139°14′ E	Oct. 25, 2005	Ns	S	Present study
15	Yamakita (Sakawa-gawa)	Kanagawa	n	n	Oct. 18, 1993	Ns	HN	Ishiwata (1997a)
16	Shiroyama (Sakai-gawa)	Kanagawa	n	n	Jun. 19, 1980	Ns	HN	Ishiwata (2004)
17	Tsukui (Sagami-gawa[e])	Kanagawa	35°36′ N	139°12′ E	Nov. 29, 2005	Ns	S	Present study
18	Fujino (Tochiya-gawa)	Kanagawa	35°38′ N	139°09′ E	Nov. 29, 2005	Ns	S	Present study
19	Nanbu (Fukushi-gawa)	Yamanashi	35°13′ N	138°25′ E	Nov. 23, 2000	Ns	S	Present study (collected by Yoshinari)
20	Sekikawa (Oishi-dam)	Niigata	n	n	2001	Ns	SN	NCRE[c]
21	Sekikawa (Ara-kawa)	Niigata	n	n	2000	Ns	SN	NCRE[c]
22	Tsunan (Sode-sawa)	Niigata	36°55′ N	138°38′ E	Oct. 30, 2005	Ns	S	Present study
23	Otari (Hime-kawa)	Nagano	n	n	June 15, 2000	IM	L	Present study (collected by Tsukuda)
24	Hakuba (Matsu-kawa)	Nagano	36°44′ N	137°49′ E	May 25, 2005	Ns	S	Present study
25	Omachi (Omachi-dam)	Nagano	n	n	2002	N	SN	NCRE[c]
26	Azumino (Nakafusa-gawa)	Nagano	36°22′ N	137°46′ E	Sept 7, 2006	Ns	S	Present study
27	Azumino (Hon-sawa)	Nagano	36°18′ N	137°47′ E	–[f]	Ns, IMs	S, N, LT	Present study (Uenishi, pers. com.)
28	Azumino (Hon-sawa)	Nagano	36°18′ N	137°45′ E	June 1, 2005	Ns	S	Present study
29	Matsumoto (Fujii-sawa)	Nagano	36°14′ N	138°01′ E	–[g]	Ns, IMs	S, N, LT	Present study
30	Matsumoto (Ashiura-sawa)	Nagano	36°13′ N	138°03′ E	2005	Ns	S	Present study
31	Matsumoto (Noma-sawa)	Nagano	36°16′ N	137°45′ E	Apr. 28, 2005	Ns	S	Present study
32	Matsumoto (Shimashimadani-gawa)	Nagano	36°11′ N	137°46′ E	Oct. 28, 2005	Ns	S	Present study
33	Matsumoto (Yakasa-gawa)	Nagano	36°11′ N	137°46′ E	Oct. 28, 2005	Ns	S	Present study
34	Matsumoto (Minamikuro-sawa)	Nagano	36°14′ N	137°49′ E	Oct. 28, 2005	Ns	S	Present study
35	Matsumoto (Koono-gawa)	Nagano	36°07′ N	137°39′ E	n	N	n	Uéno (1935)
36	Matsumoto (Toku-sawa)	Nagano	36°15′ N	137°42′ E	n	N	n	Uéno (1935)
37	Kiso (Misogawa-dam)	Nagano	n	n	2000	Ns	SN	NCRE[c]
38	Kiso (Kiso-gawa)	Nagano	35°56′ N	137°47′ E	June 22, 2005	Ns	S	Present study
39	Ina (Oguro-gawa)	Nagano	35°49′ N	137°51′ E	Aug. 4, 2005	Ns	S	Present study
40	Tenryu (Toyama-gawa)	Nagano	35°23′ N	138°05′ E	June 4, 2004	N	S	Present study (collected by Yoshinari)
41	Hakusan (Oya-dani)	Ishikawa	36°35′ N	136°47′ E	Oct. 6, 2005	Ns	S	Present study (collected by Kyuka)
42	Hakusan (Tedorigawa-dam)	Ishikawa	n	n	2002	Ns	SN	NCRE[c]
43	Toyama (Inotani-gawa)	Toyama	36°28′ N	137°13′ E	May 4, 2006	N	S	Present study
44	Tonami (Sho-gawa)	Toyama	n	n	2002	Ns	SN	NCRE[c]
45	Ono (Managawa-dam)	Fukui	n	n	2002	Ns	SN	NCRE[c]

Appendix. *Continued*

No. in Fig. 1	Locality				Collected date	Stage of collected samples[a]	Collecting method[b]	Reference
	Site	Prefecture	Latitude	Longitude				
46	Fujieda (Seto-gawa)	Shizuoka	34°58′ N	138°11′ E	June 4, 2004	IM	N	Present study (collected by Yoshinari)
47	Fujieda (Seto-gawa)	Shizuoka	34°59′ N	138°12′ E	July 6, 1997	Ns	S	Present study (collected by Torii)
48	Izu (Yoshina-gawa)	Shizuoka	34°53′ N	138°53′ E	June 27, 2005	Ns	S	Present study (collected by Turuta)
49	Shimada (Oi-gawa)	Shizuoka	n	n	2004	N	SN	NCRE[c]
50	Shitara (Togami-gawa)	Aichi	35°06′ N	137°34′ E	Mar. 26, 2006	Ns	S	Present study
51	Shitara (Togami-gawa)	Aichi	35°06′ N	137°33′ E	Mar. 26, 2006	Ns	S	Present study
52	Kasugai	Aichi	n	n	n	N	n	Kasugai City web site
53	Nakatsugawa (Nakatsu-gawa)	Gifu	35°26′ N	137°30′ E	June 10, 2005	Ns	S	Present study
54	Ibikawa (Ozu-gawa)	Gifu	35°35′ N	136°31′ E	May 13, 2002	IM	S	Present study (collected by Kawase)
55	Ibikawa (Yokoyama-dam)	Gifu	n	n	2003	N	SN	NCRE[c]
56	Kameyama (Anraku-gawa)	Mie	34°55′ N	136°23′ E	Nov. 13, 2005	Ns	S	Present study
57	Mastuzaka (Hasuchi-dam)	Mie	n	n	n	n	SN	NCRE[c]
58	Koka (Tamura-gawa)	Shiga	34°56′ N	136°21′ E	Oct. 6, 2004	Ns	S	Present study (collected by Kawase)
59	Yogo (Obanashi-gawa)	Shiga	35°39′ N	136°15′ E	Aug. 21, 2000	N	S	Present study (collected by Yoshinari)
60	Kyoto	Kyoto	n	n	n	N	n	Kyoto Prefecture web site
61	Kawakami (Otonashi-gawa)	Nara	34°21′ N	135°54′ E	Nov. 11, 2005	Ns	S	Present study
62	Kitayamakawa (Kitayama-gawa)	Nara	34°10′ N	135°59′ E	Nov. 11, 2005	Ns	S	Present study
63	Kawakami (Sannoko-gawa)	Nara	34°15′ N	136°05′ E	Nov. 12, 2005	Ns	S	Present study
64	Kawakami (Sannoko-gawa)	Nara	34°15′ N	136°05′ E	Nov. 12, 2005	Ns	S	Present study
65	Kawakami (Sannoko-gawa)	Nara	34°15′ N	136°03′ E	Nov. 12, 2005	Ns	S	Present study
66	Kawachinagano (Yamada-gawa)	Osaka	34°21′ N	135°30′ E	Sept. 14, 2005	Ns	S	Present study
67	Hashimoto (Ishi-kawa)	Wakayama	34°21′ N	135°34′ E	Sept. 14, 2005	Ns	S	Present study
68	Aridagawa (Yugawa-gawa)	Wakayama	n	n	Aug. 27, 2006	Ns, IM	n	Present study (collected by Takemon)
69	Inagawa (Tsukinami-gawa)	Hyogo	34°56′ N	135°22′ E	Apr. 15, 2006	N	S	Present study
70	Hatsukaichi (Hosomi-dani)	Hiroshima	34°33′ N	132°06′ E	Sept. 9, 2004	Ns	S	Present study (collected by Mori)
71	Hiroshima	Hiroshima	n	n	n	N	n	Hiroshima City web site
72	Kagamino (Shiraka-kawa)	Okayama	n	n	Sept. 23, 2003	N	n	Present study (collected by Mori)
73	Chizu (Chiyo-gawa)	Tottori	35°14′ N	134°21′ E	Sept. 23, 2003	Ns	S	Present study (collected by Yoshinari)
74	Naka (Naka-gawa)	Tokushima	33°50′ N	134°00′ E	May 11, 2004	Ns	S	Present study (collected by Nio)
75	Mima (Nonomuradani-gawa)	Tokushima	34°06′ N	134°04′ E	Sept. 14, 2006	Ns	S	Present study
76	Mima (Nonomuradani-gawa)	Tokushima	34°06′ N	134°04′ E	Sept. 14, 2006	Ns	S	Present study
77	Takamatsu (Uchiba-gawa)	Kagawa	34°07′ N	134°43′ E	Sept. 14, 2006	Ns	S	Present study
78	Kuma-kogen	Ehime	33°37′ N	132°55′ E	Sept. 15, 2006	Ns, IM	S, N	Present study
79	Uchiko (Ohira-gawa)	Ehime	33°36′ N	132°49′ E	Sept. 15, 2006	Ns	S	Present study
80	Uchiko (Misogi-gawa)	Ehime	33°30′ N	132°40′ E	Sept. 15, 2006	Ns	S	Present study
81	Saijo (Kamo-gawa)	Ehime	33°45′ N	133°00′ E	May 18, 2004	Ns	S	Present study (collected by Nio)
82	Shikoku-chuo (Yanase-dam)	Ehime	n	n	n	Ns	SN	NCRE[c]
83	Ino (Tera-kawa)	Kochi	n	n	Oct. 10, 2004	N	S	Present study (collected by Nio)
84	Ino (Takadaru-gawa)	Kochi	33°38′ N	133°01′ E	Mar. 16, 2004	Ns	S	Present study (collected by Nio)
85	Tsuno (Shimanto-gawa)	Kochi	33°26′ N	133°05′ E	Sept. 5, 2000	Ns	S	Present study (collected by Torii)
86	Niyodogawa (Iwaya-gawa)	Kochi	n	n	Sept. 11, 2004	N	S	Present study (collected by Taniguchi)
87	Niyodogawa (Odo-dam)	Kochi	n	n	n	Ns	SN	NCRE[c]
88	Kochi (Anagawa-gawa)	Kochi	n	n	Feb. 10, 2004	Ns	S	Present study (collected by Nio)
89	Tosa (Akuta-gawa)	Kochi	33°42′ N	133°00′ E	May 27, 2004	Ns	S	Present study (collected by Nio)
90	Tosa (Seto-gawa)	Kochi	33°44′ N	133°22′ E	May 27, 2004	IM	S	Present study (collected by Nio)
91	Shimanto (Yasuhara-gawa)	Kochi	33°17′ N	132°05′ E	May 8, 2004	Ns	S	Present study (collected by Nio)

Appendix. *Continued*

No. in Fig. 1	Locality				Collected date	Stage of collected samples[a]	Collecting method[b]	Reference
	Site	Prefecture	Latitude	Longitude				
92	Sukumo (Nakasujigawa-dam)	Kochi	n	n	n	Ns	SN	NCRE[c]
93	Sasaguri	Fukuoka	33°38′ N	130°33′ E	May 10, 2006	Ns	S	Present study
94	Sasaguri	Fukuoka	33°38′ N	130°33′ E	May 10, 2006	Ns	S	Present study
95	Kama (Onga-gawa)	Fukuoka	33°28′ N	130°48′ E	May 10, 2006	Ns	S	Present study
96	Hita (Kagetsu-gawa)	Oita	33°22′ N	130°58′ E	May 10, 2006	Ns	S	Present study
97	Kokonoe (Naruko-gawa)	Oita	33°09′ N	131°13′ E	May 11, 2006	Ns	S	Present study
98	Taketa (Seri-kawa)	Oita	33°03′ N	131°23′ E	May 11, 2006	Ns	S	Present study
99	Bungo-ono	Oita	n	n	n	n	n	Present study
Korea								
100	Chiak-san	Kangwon-do	n	n	May 19, 2000	N	S	Present study (collected by Nozaki)

n, exact reading not taken
[a] In regard to stage of collected samples: nymph (N), subimago and imago (IM); when multiple individuals were collected, Ns or Ims
[b] In regard to collection methods, four categories: searching or looking (S), netting (N), using hand net (HN), server net (SN), and light trapping (LT)
[c] NCRE, the "National Censuses on River Environments" project
[d] As recorded on Tokura Dam Construction Office, Incorporated, Administrative Agency, Japan Water Agency, web site
[e] A small branch of the Sagami-gawa River
[f] May 25, 2005, and other days from June 9, 2005 to Sept. 13, 2006
[g] Sept. 23, 2004, and other days from Oct. 8, 2005 to June 23, 2006