

## Prediction of the emergence period for overwintering chironomid larvae (Diptera: Chironomidae) at the lower reaches of the Kiso River, Central Japan

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**Abstract:** Species composition and period of emergence were investigated for overwintering Chironomidae at the lower reaches of the Kiso River by rearing larvae to obtain fundamental information for drafting a plan to prevent outbreaks of nuisance midges. Adults of 9 species emerged from the substrate samples in February 2006. The four most abundant species, *Stictochironomus akizukii*, *Hydrobaenus kondoi*, *Tanytarsus takahashii* and *Einfeldia dissidens*, occupied 88.0% of the total abundance. Using effective accumulative temperature for emergence and *in situ* water temperatures, the *in situ* emergence periods of the overwintering generation in 2006 were predicted as until early March for *H. kondoi*, from the end of March to early May for *S. akizukii* and *T. takahashii*, and late April to mid-June for *E. dissidens*.

Key words: Chironomidae, emergence period, prediction, winter, Kiso River, larval rearing

### INTRODUCTION

Chironomid midges emerge in masses causing severe sanitary and economical damages in some regions (Tabaru et al., 1987; Ali, 1995). Clarification of the life cycle, especially the emergence period, is essential for drafting a plan to control outbreaks of these aquatic pest insects. In general, temperature is one of the most effective environmental factors that determines emergence and the number of generations of an insect each year (Menzie, 1981; Rossaro, 1991; Tokeshi, 1995; Reynolds and Benke, 2005). Effective accumulative temperature (EAT), the cumulative temperature above the developmental threshold during the period from the start of rearing to adult emergence, allows

prediction of the length of the immature stage and time of adult emergence under variable temperature conditions in the field (Gullan and Cranston, 1994; Kondo, 1996).

Most of the nuisance chironomids emerge from spring to autumn (e.g. Nakazato et al., 1998; Hirabayashi et al., 2003). However, two Orthoclaadiinae species, *Hydrobaenus kondoi* Sæther and *H. kisoecundus* Sasa et Kondo emerge in large numbers during the winter at the middle and lower reaches of the Kiso River, Central Japan (Kondo and Sasa, 1994; Kondo, 1996, 2001). Since 1986, outbreaks of these two species, especially *H. kondoi*, have been reported almost every year upstream of the Kisogawa-ohseki roller gate dam, which was constructed in 1977 (Kondo, 2001). The outbreaks are

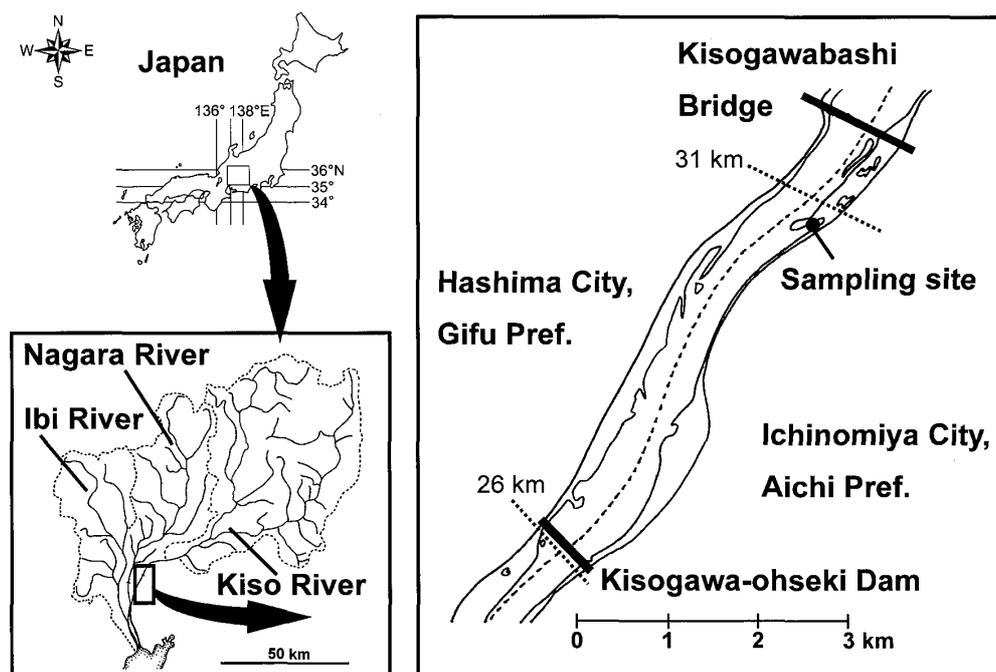


Fig. 1. Map of the Kiso River showing the location of the sampling site.

thought to be associated with a decrease in current, accumulation of detritus and changes in particle size composition of the substrate caused by the dam (Kondo, 2001).

In relation to chironomid fauna of the Kiso River, most attention has focused on the outbreak of two *Hydrobaenus* species, especially *H. kondoi* in the 1990s (e.g. Sæther, 1989; Kondo, 1991, 1996; Sasa and Kondo, 1991). At the same time, other species have been recorded from the basin and biological observations made (Sasa and Kondo, 1991, 1993, 1994; Kondo and Sasa, 1994). Kondo and Sasa (1994) reported the emergence period of 19 species collected by light traps and emergence traps at the lower reaches of the river. However, no faunal investigation of chironomids has been conducted at least during the last 10 years at the Kiso River. Long term changes in species composition were observed at many other basins as well as environmental conditions (e.g. Nishino, 2001; Kawai et al., 1997). Continuous faunal monitoring is needed to predict and control outbreaks of other species at the lower reaches of the Kiso River. One of the first steps is to understand the current conditions of *in situ* representa-

tive fauna, i.e. composition of dominant species and their period of emergence.

The objectives of our study were to elucidate the species composition of the major overwintering chironomids other than the two *Hydrobaenus* species and their emergence periods at the lower reaches of the Kiso River. We collected riverbed substrates for larval rearing in February 2006 at a representative site where many complaints were made about nuisance chironomid midges. Temperatures of rearing water and the numbers of emerging adults were recorded every day to estimate the *in situ* period of emergence of the dominant species using EAT needed for emergence during rearing and the *in situ* water temperature. Finally, possibility of future outbreaks of chironomids other than the *Hydrobaenus* species at the region is discussed.

## MATERIALS AND METHODS

### *Chironomid sampling and rearing*

The Kiso River is the largest river among "three rivers in Kiso," followed by the Nagara and Ibi Rivers. The Kiso River originates from Mt. Hachimori (2,446 m a.s.l.) of the Southern Hida Mountains and

stretches for 229 km with 5,275 km<sup>2</sup> of catchment area. The sampling site (35° 17' N, 136° 43' E) was located near the left bank of the lower reaches of the Kiso River (Higashikaganoi, Ichinomiya City, Aichi Prefecture, Japan), 31 and 4 km upstream from the river mouth and Kisogawa-ohseki Dam, respectively (Fig. 1). The width of the river was approximately 500 m and the mean depth was approximately 1.0 m in this area. The current was very slow and stagnant so the landscape of the site looked like an artificial lake with a highly uniform environment. For larval rearing, riverbed substrates were collected twice with an Ekman-Birge grab sampler (opening: 15 cm × 15 cm; depth: 5–6 cm) in a littoral area on 9 February 2006. Depth of the water at the sampling point was 30 cm and temperature of the substrate was 5.8°C during the sampling. The substrate consisted of sandy mud and a little plant debris.

The two substrate samples were mixed in a plastic bag and transported to our laboratory under cool conditions on 10 February 2006. Then they were transferred to a cylindrical plastic bottle (diameter 10 cm × height 25 cm) with an adequate amount of water collected from the site (up to 10 cm from the opening). Moderate aeration was supplied during rearing with a diaphragm pump through an air stone. The opening of the bottle was covered with fine nylon net to prevent escape of emerging adults. The samples were reared at room temperature (8.2–26.6°C), higher than at the collection site, to facilitate emergence. Temperature of the rearing water was monitored every 1 hour during rearing with a thermistor thermometer (TR-71S; T and D Corp.) and ranged between 6.5 to 25.2°C (Fig. 2). Additional food was not supplied during rearing because the substrate sample contained enough detritus as food for the chironomid larvae. Emerging adults and pupal exuviae were checked and collected every day for 102 days until 23 May 2006,

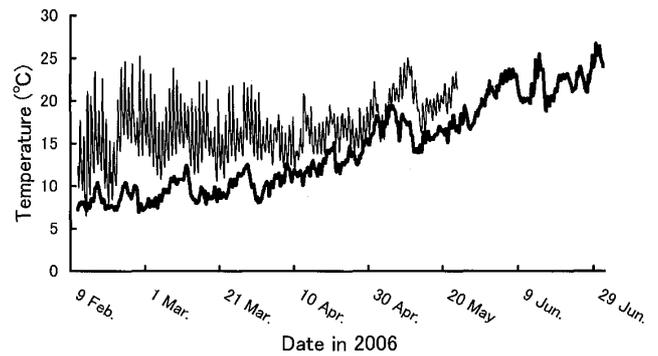


Fig. 2. Temperature of rearing water (light line) and *in situ* river water (bold line).

15 days after the last emergence ceased. At the end of the rearing, the substrates were sieved through a 418 µm mesh net (NGG42) to collect the remaining larvae. The adults and remaining larvae were preserved in 70% ethanol from the date collected until identification was conducted.

#### Identification of species

All the adults and remaining larvae were mounted on slides with gum chloral under a binocular dissecting microscope. Male adults were observed under a high-powered microscope and identified to species following the taxonomical keys of Pinder (1978), Wiederholm (1989), Sasa and Kikuchi (1995) and Sæther et al. (2000). Female adults were similarly identified to genus then associated with the males. The remaining larvae were identified to genus following Wiederholm (1983) and Sæther et al. (2000).

#### Estimation of in situ emergence period

Developmental thresholds for the dominant species are  $-2.17^{\circ}\text{C}$  for *H. kondoi* (Kondo, 1996) and  $5^{\circ}\text{C}$  for Chironominae species (Shirota, 1969; Hauer and Benke, 1991; Reynolds and Benke, 2005). EAT from the beginning of the rearing to emergence was calculated individually by the following equation:

$$\text{EAT} = \sum_{i=1}^n (t_i - T)$$

where  $t_i$  is mean water temperature on  $i$ -th day after the sampling,  $T$  is the developmental threshold of the species and  $n$  is

Table 1. A list of chironomid species, abundance, density and proportion collected at the sampling site.

Species	No. of adults emerged*	Proportion (%)
Prodiamesinae		
<i>Monodiamesa</i> sp.	F1	1.3
Tanypodinae		
<i>Procladius choreus</i> (Meigen, 1804)	M2, F1	4.0
Orthoclaadiinae		
<i>Hydrobaenus kondoi</i> Sæther, 1989	M10, F8	24.0
Chironominae		
Chironomini		
<i>Einfeldia dissidens</i> (Walker, 1851)	M5, F6	14.7
<i>Polypedilum cultellatum</i> Goetghebuer, 1931	M1	1.3
<i>Polypedilum</i> sp.	L3	4.0
<i>Stictochironomus akizukii</i> (Tokunaga, 1940)	M13, F6	25.3
Tanytarsini		
<i>Tanytarsus oyamai</i> Sasa, 1979	M1	1.3
<i>Tanytarsus takahashii</i> Kawai et Sasa, 1985	M7, F11	24.0
Total 9 species	M39, F33, L3	100.0

\*M, male adults; F, female adults; L, larvae remaining at the end of rearing.

the day of emergence of the individual adult. Thus, EAT obtained in this study does not indicate requirements to complete development from egg to adult. Using the water temperature of the river measured at the nearest public observatory, Nannoh-ohashi Station (Ministry of Land, Infrastructure and Transport, Government of Japan, 2006) (Fig. 2), located 4.2 km west-southwest of the sampling site, we estimated the *in situ* emergence period for the dominant species. The date when the EAT of an individual calculated in our study was achieved at *in situ* water temperature was regarded as the *in situ* day of emergence. The *in situ* emergence period of a species was regarded as between the earliest and the latest estimated date of emergence among individuals of the species derived from the minimum and maximum EAT.

## RESULTS

A total of 39 males, 33 females and 3 remaining larvae belonging to 9 species were collected (Table 1). *Polypedilum* sp. did not emerge during rearing but live larvae remained and were identified by sieving at the end of the rearing.

Table 2. Days and effective accumulative temperatures (EAT) during larval rearing of the four most abundant species needed for emergence.

Species	n	Needed for emergence	
		Days*	EAT (°C·day)*
<i>H. kondoi</i>	18	7.4 ± 4.0 <sup>a</sup>	103.1 ± 65.8 <sup>a</sup>
<i>T. takahashii</i>	18	28.2 ± 8.1 <sup>b</sup>	288.1 ± 87.9 <sup>b</sup>
<i>S. akizukii</i>	19	29.6 ± 7.8 <sup>b</sup>	304.0 ± 85.0 <sup>b</sup>
<i>E. dissidens</i>	11	69.6 ± 15.3 <sup>c</sup>	744.9 ± 171.0 <sup>c</sup>

\*Values (mean ± SD) in the same column with different superscripts (a, b, c) are significantly different from each other (Tukey-Kramer test,  $p < 0.01$ ).

Note that the values do not indicate the requirements to complete development from egg to adult.

Chironominae was the most abundant subfamily accounting for 70.7% of the total abundance, followed by Orthoclaadiinae (24.0%), Tanypodinae (4.0%) and Prodiamesinae (1.3%). The four most abundant species, *Stictochironomus akizukii* (Tokunaga) (25.3%), *H. kondoi* (24.0%), *Tanytarsus takahashii* Kawai et Sasa (24.0%) and *Einfeldia dissidens* (Walker) (14.7%), occupied 88.0% of the total abundance.

Emergence periods were different

Table 3. Estimated emergence period in 2006 at the sampling site of the four most abundant species.

Species	n	Estimated emergence date			
		Days after Feb. 10*	Earliest	Mean	Latest
<i>H. kondoii</i>	18	10.3±6.2 <sup>a</sup>	11 Feb.	20 Feb.	3 Mar.
<i>T. takahashii</i>	18	61.0±12.3 <sup>b</sup>	28 Mar.	12 Apr.	3 May
<i>S. akizukii</i>	19	63.2±11.6 <sup>b</sup>	28 Mar.	14 Apr.	5 May
<i>E. dissidens</i>	11	104.3±13.7 <sup>c</sup>	23 Apr.	25 May	10 Jun.

\*Values (mean±SD) with different superscripts (a, b, c) are significantly different from each other (Tukey-Kramer test,  $p < 0.01$ ).

during the rearing among the species (Table 2). Among the four most abundant species, *H. kondoii* emerged soon after the beginning of rearing (1–14 days), significantly earlier than the other three species (Tukey-Kramer test,  $p < 0.01$ ). On the other hand, *E. dissidens* emerged significantly later (34–87 days) than the other species ( $p < 0.01$ ). Emergence of *T. takahashii* (19–43 days) and *S. akizukii* (19–46 days) occurred during a similar period between *H. kondoii* and *E. dissidens* ( $p < 0.01$ ).

Similarly, the EAT of *H. kondoii* was the lowest (Tukey-Kramer test,  $p < 0.01$ ) and of *E. dissidens* was the highest among the four species ( $p < 0.01$ ) (Table 2). *Tanytarsus takahashii* and *S. akizukii* required almost the same EAT for emergence and both occurred between *H. kondoii* and *E. dissidens* ( $p < 0.01$ ).

Based on the EAT values and *in situ* water temperatures, *in situ* emergence of *H. kondoii* was estimated to cease in early March at the lower reaches of the Kiso River in 2006 (Table 3). Emergence periods for both *T. takahashii* and *S. akizukii* in 2006 were expected to be from the end of March to early May, mainly mid-April. *Einfeldia dissidens* was estimated to emerge from late April to mid-June in 2006, mainly late May.

## DISCUSSION

*In situ* emergence periods of the four most abundant species estimated in our study are based on the results of rearing of benthic chironomid larvae collected in the

winter. Therefore, the estimated emergence periods are applicable to only overwintering generations of the species. Several studies have reported the emergence periods of the four species in the field. *Hydrobaenus kondoii* shows massive emergence twice a year, in December and February, at the Kiso River (Kondo, 1996). In addition, massive swarms of *H. kondoii* were found at the sampling sites during our substrate collection (Hirabayashi et al., personal observation). *Stictochironomus akizukii* shows its emergence peak in spring and autumn from Lake Kawaguchi (Hirabayashi, 2001) and mainly in September from Lake Suwa (Hirabayashi et al., 2003). *Tanytarsus takahashii* emerges mainly in May to July from the Nagara River (Kawai et al., 1997) and in June to September from Lake Suwa (Hirabayashi et al., 2003). *Einfeldia dissidens* emerges mainly in May to October from Lake Suwa (Nakazato et al., 1998; Hirabayashi et al., 2003). Therefore, both the order of emergence during rearing and the *in situ* emergence of the four species estimated in this study almost correspond well to those reported in the field. This indicates that the emergence periods of the four species estimated in our study are reliable so that EAT values during rearing and *in situ* water temperatures are useful to predict emergence period of nuisance chironomid species, even if the emergence period has not been reported. Emergence periods should vary due to differences in *in situ* water temperature fluctuations (Rossaro, 1991) among regions and years, and larval instar composition of overwintering gen-

erations among the species.

Emergence period should be a reflection of the *in situ* composition of immature stages. However, since immature development greatly depends on water temperature (Gullan and Cranston, 1994), EAT obtained in the present study for an individual to emerge during rearing reflects the initial developmental stage. The EAT values calculated in our study were applied for estimation of emergence period at the sampling site in 2006. In order to estimate *in situ* larval instar composition, further studies of chironomid species on EAT are necessary to clarify the EAT for the complete life cycle from egg hatch to adult emergence.

Although emergence traps and light traps are direct methods to investigate *in situ* emergence period of chironomids in the field, the precision depends on the sampling frequency. In addition, these methods are often susceptible to weather or differences in the degree of adult phototaxis among species. On the other hand, our larval rearing and EAT is an indirect method, but it can estimate *in situ* emergence dates of individuals from a few substrate samples. Emerging adults can be collected every day in the laboratory instead of from the field. Therefore, emergence period can best be estimated in a uniform area by larval rearing then by intermittent samplings using emergence traps and light traps.

The differences in emergence ratio and mortality among species and instars under the rearing conditions may have distorted chironomid assemblage data obtained in our study from *in situ* larval assemblages. *Polypedilum* sp. remained as larvae and did not emerge throughout the rearing period of 102 days. Although we confirmed by sieving that no dead larvae remained at the end of rearing, small larvae and decayed carcasses may have passed through the mesh. Nevertheless, 72 emerging adults and 3 remaining larvae, i.e.  $37.5 \text{ ind.} \cdot 0.0225 \text{ m}^{-2}$  in total, were collected in our study. This total abundance

is very similar to the *in situ* larval density (mean  $43.7 \text{ ind.} \cdot 0.0225 \text{ m}^{-2}$ ) at the same site in December in a study by Kondo (2001). Therefore, the majority of larvae and pupae of the dominant species can be regarded as successfully emerging during rearing.

Despite almost the same sampling site, our results were different from Kondo and Sasa (1994) except that *H. kondo* was abundant in the winter at the Kiso River. In our study, the relative abundance of Chironominae was much higher than that of Kondo and Sasa (1994) collected by an emergence trap in February (59.7%). We also collected fewer *H. kondo* (29.9% by Kondo and Sasa, 1994) and no *H. kisoecundus* (7.5%). Differences in sampling methods may be the major cause because our larval rearing method allows detection of species that do not emerge in the winter. Nevertheless, considering that the major larval habitats of *S. akizukii*, *T. takahashii* and *E. dissidens* are rather fine sediments in slow flowing or standing waters (Wiederholm, 1989; Nakazato et al., 1998), our results suggest that substrate conditions have changed at the sites during the last 10 years. Decrease in *Hydrobaenus* species and increase of Chironominae both emphasize the long term succession of chironomid assemblages.

Recently, *H. kondo* is the only species from the species collected in our study reported nuisance complaints along the middle to lower reaches of the Kiso River. *Stictochironomus akizukii* and *T. takahashii* were also abundant at the sampling site, but they have not been reported to emerge at nuisance levels elsewhere (Tabaru et al., 1987; Ali, 1995). *Einfeldia dissidens* emerges massively from June to September and is one of the nuisance species at the littoral area of Lake Suwa (Hirabayashi et al., 2003). The larval habitat of *H. kondo* is mainly slow current rivers where middle sized sand and organic matter are accumulated (Kondo, 2001). In Lake Suwa, *E. dissidens* inhabits the

littoral zone of sandy mud substrates (Nakazato et al., 1998). Both the increase in the relative abundance of *E. dissidens* and similarity of habitat preferences between *H. kondoii* and *E. dissidens* suggest that massive emergence of *E. dissidens* could occur with environmental changes, e.g. changes in particle size composition of the substrate. Near the sampling site, accumulation of fine detritus and sand has increased due to a decrease in the current (Kondo, 2001). Further monitoring of both environmental conditions and chironomid assemblages, and interspecific relationships among species with different emergence periods, would help to predict and control future outbreaks of nuisance species other than *H. kondoii*.

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