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Source: Environmental Entomology, 41(4):950-958. 2012.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/EN11286
URL: http://www.bioone.org/doi/full/10.1603/EN11286

Development of *Glyptotendipes tokunagai* (Diptera: Chironomidae) Under Different Temperature Conditions

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Environ. Entomol. 41(4): 950–958 (2012); DOI: http://dx.doi.org/10.1603/EN11286

ABSTRACT The nonbiting midge, *Glyptotendipes tokunagai* Sasa (Diptera: Chironomidae), is an organism that can be used as a water quality indicator. Development of this insect under different temperatures was evaluated. The highest egg hatching rate was 99.12 ± 1.47% at 30°C, and the lowest was 84.50 ± 11.09% at 10°C. No eggs hatched at temperatures of 8 and 42°C. Developmental rate (1/h) increased gradually as temperature increased from 10 to 35°C with a peak rate (0.045 ± 0.0079) at 35°C. The estimated lower thermal threshold for egg hatching was 9.3°C. Adult emergence rate was highest (80.6%) at 30°C and lowest (22.2%) at 15°C. The developmental times (egg to adult) for both males and females decreased from 2,102.6 to 457.2 h and from 2,337.1 to 619.8 h as temperatures increased from 10 to 30°C. The estimated lower thermal thresholds for males and females were 11.4 and 10.4°C, respectively. The body sizes of all *G. tokunagai* decreased as temperature increased from 15 to 35°C. From the results of this study, the approximate rearing temperature for *G. tokunagai* is suggested to be 25–30°C for egg hatching, larval development, and emergence. Our results demonstrate that *G. tokunagai* is a potential test insect species that can be reared in the laboratory by providing optimal temperature conditions.

KEY WORDS nonbiting midge, degree-day, developmental rate, thermal threshold, toxicity test organism

The dipteran family Chironomidae, or nonbiting midges, among aquatic organisms has been used worldwide as a water quality indicator (Weltje et al. 2010). In accordance with the guidelines established by the Organization for Economic Cooperation and Development (OECD), only three chironomid species, *Chironomus riparius* Meigan, *C. tentans* Fabricius, and *C. yoshimatsui* Martin and Sublette, have been used as standard organisms for toxicity testing, and the biology, life cycle characteristics, laboratory maintenance methods, and responses to environmental changes of these organisms have been fairly well-elucidated (U.S. EPA 1994, Watts and Pascoe 2000, OECD 2001). Although these three species are most frequently used for toxicity tests, there remains a clear need for new biological indicator species to be identified for diverse environmental uses, including environmental monitoring and ecological risk assessment. Biological data of the new indicator species also can be used as reference data for those of other indicator species with which to make interspecific comparisons.

The chironomid midge *Glyptotendipes tokunagai* Sasa is distributed broadly throughout East Asia and is frequently a dominant aquatic insect species in organic rich urban streams (Sasa 1989, Lee et al. 2009). This species has a short life cycle, and can be easily cultured in the laboratory. Although some aspects of *G. tokunagai* biology have been studied by some researchers (Kawai and Konishi 1986, Yano et al. 1991), a comprehensive temperature-dependent experiment under laboratory conditions is crucial to establish a protocol including low developmental threshold temperature as well as high developmental inhibition temperature for culturing the species. The effects of temperature on insect development are relevant to a variety of factors, including mortality, developmental time, and body sizes (Sweeney 1984, Frouz et al. 2002). In this study, the development of *G. tokunagai* egg and immature stages were investigated under a variety of temperature conditions.

Materials and Methods

Collecting and Rearing. In April 2007, four *G. tokunagai* egg masses were collected from Jungrang stream, an urban stream that branches off the Han River in Seoul, Korea. The egg masses were quickly transferred to the laboratory and reared in petri dishes (diameter 150 and height 20 mm) with ≈100 ml of preaerated distilled water and fine sand for building the case. Tetramin (<0.2 mm) (TetraWerke, Melle, Germany) dissolved in preaerated distilled water was provided as feed for the larvae. Fourth instar larvae or

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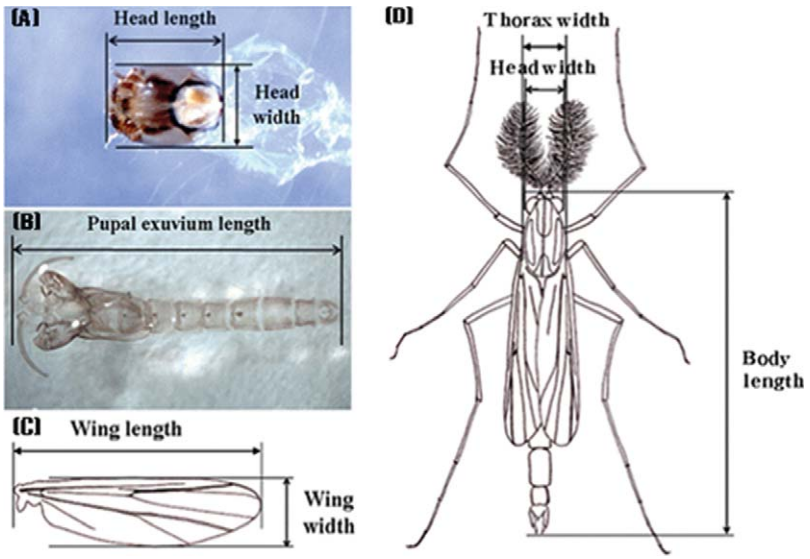

Fig. 1. Measurement of larva (head length and width) (A), pupa (exuvium length) (B), wing (length and width) (C), and adult body (body length, head width, and thorax width) (D) of *G. tokunagai*.

pupae were transferred to a plastic container (length 250, width 270, and height 170 mm) containing preaerated distilled water (depth 40–50 mm) and fine sand (depth 30–40 mm), and then placed inside a rearing cage (acrylic box with attached screen: length 420, width 550, and height 420 mm) for emerging adults. Emerged adults were transferred to a mating cage (cylindrical acrylic container: diameter 120 and height 210 mm) containing ≈300 ml of preaerated distilled water for oviposition. Reared individuals were discriminated generation by generation. Rearings were distinguished and designated by generation.

Egg Development. Eggs laid at the second and third generations were used for this experiment. Eighty-four egg masses (2–10 egg masses at each temperature) were reared in a petri dish (diameter 150 and height 20 mm) with ≈100 ml of preaerated distilled water at 15 constant temperatures ranging from 8 to 42°C (increments of 2–3°C) with a photoperiod of 16:8 (L:D) h and a light intensity of 1,500 lux. All petri dishes were randomly placed in either vertical or horizontal orientations within the incubator. Egg hatching was checked every 8 h.

Larval Development. The temperature range for the larval development experiments was determined based on the egg development results. Six third generation egg masses were dissected with a pipette. In total, 144 eggs were transferred to 12-well cell culture plates (SPL Lifescience, Seoul, Korea), one egg per well, with 1 ml of preaerated distilled water. The 12-well cell culture plates were incubated at temperatures of 10, 15, 20, 25, 30, 35, and 40°C, respectively, under a photoperiod of 16:8 (L:D) h and a light intensity of 1,500 lux. The well dimensions and working volume of the 12-well cell culture plates were 22.1 mm in diameter and 2 ml, respectively. Dissolved Tetramin (<0.2 mm) was supplied as food. All developmental changes such as molting to the next instar or next stage (larva, pupa, and adult) were recorded every 24 h. Emerged adults and fourth instar and pupal exuviae were preserved in 80% ethyl alcohol.

Body Size Measurement. Dimensions of the last instar larvae (head capsule width and length), the pupal exuvium (length), and the adults (head width, thorax width, wing width and length, and body length) were measured as indicated in Fig. 1 using a stereoscopic microscope (Carl Zeiss Stemi 2000-C, Oberkochen, Germany). All measurements were obtained from captured computer images using an image analyzer (AxioVision Rel. 4.5 program, Carl Zeiss).

Data Analysis. An analysis of variance (ANOVA) was used at a significance level of 0.05 to determine the

Table 1. Number of egg masses, mean no. of eggs, and hatching rate (%) of *G. tokunagai* under constant temperatures

Temperature (°C)	No. of egg masses	Mean no. of eggs in an egg mass	Hatching rate (%)
8	6	372.0 ± 290.3	0
10	5	637.8 ± 250.8	84.5 ± 11.1
13	5	674.2 ± 145.1	85.4 ± 17.0
15	5	655.4 ± 160.8	88.4 ± 10.5
17	5	758.8 ± 143.1	98.8 ± 0.9
20	6	801.2 ± 207.3	97.3 ± 1.6
22	5	757.4 ± 150.6	97.6 ± 2.3
25	5	758.0 ± 86.5	97.3 ± 4.1
28	6	855.3 ± 184.2	96.7 ± 3.6
30	5	918.0 ± 282.9	99.7 ± 1.5
33	5	919.2 ± 216.1	97.8 ± 3.7
35	10	817.1 ± 178.6	88.1 ± 26.2
38	6	908.5 ± 130.3	92.2 ± 26.6
40	8	756.3 ± 168.0	90.5 ± 11.2
42	2	399.0 ± 257.4	0

Eggs were not hatched at 8 and 42°C (mean ± SD).

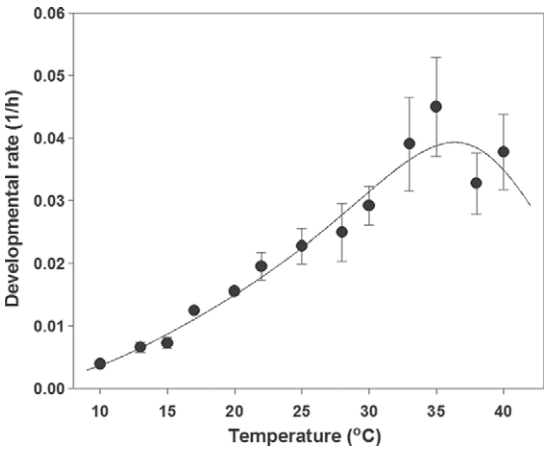

Fig. 2. Temperature-dependent developmental rate (1/h) curve for *G. tokunagai* eggs at various temperatures. Vertical lines indicate the SD of the mean.

effect of temperature on developmental time (h), developmental rate (1/h), and body sizes (mm). Developmental times of the same stage were statistically tested by ANOVA with Tukey's test. Additionally, developmental time and body sizes were separately tested at each temperature for each gender using *t*-tests at the significance level of 0.05 (SAS Institute 1999). Linear regression analysis was used to estimate the lower developmental threshold temperature. To explain the effect of mean developmental rates under various temperature conditions, a modification of the model developed by Sharpe and DeMichele (1977) as presented by Schoolfield et al. (1981) was used to show the high temperature inhibition. The parameters were estimated using the SAS program provided by Wagner et al. (1984). The relevant equation is expressed as follows:

$$r(T) = \frac{RHO25\,\frac{T}{298.15}\exp\left[\frac{HA}{R}\left(\frac{1}{298.15}-\frac{1}{T}\right)\right]}{1+\exp\left[\frac{HL}{R}\left(\frac{1}{TL}-\frac{1}{T}\right)+\exp\left[\frac{HH}{R}\left(\frac{1}{TH}-\frac{1}{T}\right)\right]\right]}$$

where $r\,(T)$ is the developmental rate at temperature T (Kelvin temperature), R is the universal gas constant (1.987 cal degree · mol), *RHO*25 is the developmental rate at 25°C, assuming no enzyme inactivation, and *HA* is the enthalpy of activation of the reaction catalyzed by a rate-controlling enzyme, *TL* is the Kelvin temperature at which the rate-controlling enzyme is half active and half low-temperature inactive, *HL* is the change in enthalpy associated with the low-temperature inactivation of the enzyme, *TH* is the Kelvin temperature at which the rate-controlling enzyme is half active and half-high temperature inactive, and *HH* is the change in enthalpy associated with the high-temperature inactivation of the enzyme.

Results

Egg Development. The mean number of eggs (±SD) in a *G. tokunagai* egg mass was 775.9 ± 196.3 (range, 211–1,318). Egg hatching rate was the highest at 30°C and the lowest at 10°C, and eggs did not hatch at eight or 42°C. As temperatures decreased to <15°C, hatching rates fell to <90%. Development time was significantly influenced by temperature (ANOVA, $P < 0.05$; Table 1). As temperature increased from 10 to 35°C, the developmental rate gradually increased up to 0.045 ± 0.0079 (Fig. 2). In the high-temperature range over 35°C, however, the developmental rate declined. As a result, the developmental rate generally fit well with the nonlinear biophysical development model within all temperatures tested (Table 2; Fig. 2). The lower developmental threshold temperature for the egg was estimated to be 9.3°C (Table 3).

Table 2. Parameter estimates and *r* (T) values for the developmental rate function (Schoolfield et al. 1981) fitted to the mean rate vs constant data for each stage of *G. tokunagai*

Sex	Life stage	*RHO*25[a]	*HA*[b]	*TH*[c]	*HH*[d]	*TL*[e]	*HL*[f]
Male	Egg	0.023	12,006.9	313.7	56,899.6	283.2	−43,282.2
	First instar	0.009	19,167.3	307.9	92,639.3	—	—
	Second instar	0.014	19,475.0	308.1	103,763.0	—	—
	Third instar	0.010	20,328.8	308.1	747,237.6	—	—
	Fourth instar	0.006	25,047.1	300.9	34,704.7	—	—
	Pupa	0.024	11,349.3	1,000.0	—	—	—
	Total	0.002	19,012.6	307.6	62,233.4	—	—
Female	Egg	0.023	12,006.9	313.7	56,899.6	283.2	−43,282.2
	First instar	0.009	19,619.2	308.1	72,245.8	—	—
	Second instar	0.014	20,397.7	308.1	114,387.3	—	—
	Third instar	0.009	20,843.7	307.0	73,803.7	—	—
	Fourth instar	0.002	8,148.6	1,000.0	—	—	—
	Pupa	0.035	20,050.5	305.8	40,458.3	—	—
	Total	0.001	14,561.9	308.2	666,394.1	—	—

[a] *RHO*25 is the developmental rate at 25°C.
[b] *HA* is the enthalpy of activation of the reaction catalyzed by a rate-controlling enzyme.
[c] *TH* is the Kelvin temp at which the rate-controlling enzyme is half active and half-high temp inactive.
[d] *HH* is the change in enthalpy associated with the high-temp inactivation of the enzyme.
[e] *TL* is the Kelvin temp at which the rate-controlling enzyme is half active and half low-temp inactive.
[f] *HL* is the change in enthalpy associated with the low-temp inactivation of the enzyme.

Table 3. Low developmental threshold temperatures for each stage and gender of *G. tokunagai*

Stage	Low developmental threshold temperatures (°C)	
	Male	Female
Egg	9.3	9.3
First instar	12.3	12.2
Second instar	12.6	13.1
Third instar	13.3	12.3
Fourth instar	10.1	8.3
Pupa	10.3	10.3
Adult	11.4	10.4

Larval Development. *G. tokunagai* larval mortality (%) at each temperature is shown in Fig. 3. Egg hatching was observed at 10 and 40°C, but the hatched first instar larvae did not survive to the following stage. Mortality decreased as temperature increased up to 30°C, and subsequently increased at 35°C. The highest emergence rate was at 30°C, and the lowest was at 15°C. The survival rates of each instar at the five tested temperatures were the lowest during the first instar periods, particularly at 35°C, and the survival rates of the first instar at the five tested temperatures varied from 59.7 to 94.4%. The gender ratios (no. females/total no. adults) at each temperature (15, 20, 25, 30, and 35°C) were estimated to be 25.0, 39.6, 39.0, 52.6, and 42.9%. The development times of males and females in the third instar (except for 15°C) ($t = -4.381$--2.321; df = 61-99; $P < 0.05$), fourth instar ($t = -16.462$--3.133; df = 27-99; $P < 0.05$), and total stages (larva to adult) ($t = -18.287$--2.506; df = 27-99; $P < 0.05$) differed significantly at all temperatures. As temperature increased from 15 to 30°C, the development time from the first instar larva to adult stage of both males and females decreased, whereas at 35°C it increased (ANOVA, $P < 0.05$). The longest development time for *G. tokunagai* of both genders was noted in the fourth instar stage (Tables 4 and 5).

The *G. tokunagai* developmental rate generally fit well into the nonlinear biophysical developmental model within all tested temperatures (Table 2 and Figs. 4A,B). The estimated lower threshold temperatures for the male and female adults were 11.4 and 10.4°C, respectively (Table 3). The lower threshold temperatures for both males and females did not differ significantly ($t = 0.561$; df = 12; $P = 0.585$).

Body Size Measurement. All body sizes, including those of the fourth larval exuviae, pupal exuviae, and adults of both genders became significantly smaller with increases in temperature from 15 to 35°C (ANOVA, $P < 0.05$; Figs. 5 and 6). The body sizes of males were smaller than those of females at all temperatures (t-test; $P < 0.05$) with the exception of the

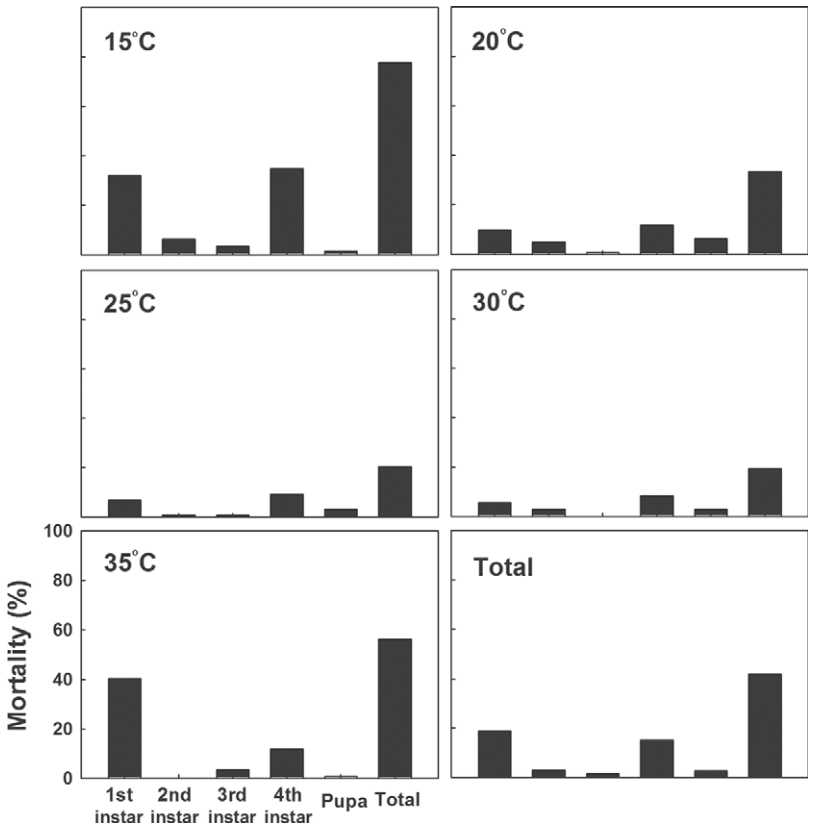

Fig. 3. Mortality (%) for each *G. tokunagai* developmental stage at five constant temperatures.

Table 4. Developmental time (T) and degree-day (D) for *G. tokunagai* male under five constant temperatures (mean ± SD)

Temperature (°C)	n^a		First instar (h)	Second instar (h)	Third instar (h)	Fourth instar (h)	Pupa (h)	Larva to adult period (h)	Egg to adult period (h)[b]
15	24	T	339.7 ± 44.3a	249.0 ± 42.9a	416.0 ± 114.7a	851.0 ± 138.5a	107.0 ± 23.5a	1,962.7 ± 178.2a	2,102.6 ± 178.2a
		D	38.2 ± 5.0	24.9 ± 4.3	29.5 ± 8.1	173.7 ± 28.3	21.0 ± 4.6	294.4 ± 26.7	33.2
20	58	T	199.2 ± 29.9b	129.9 ± 30.8b	175.9 ± 39.3b	392.7 ± 80.9b	57.9 ± 14.2b	955.6 ± 97.9b	1,019.9 ± 97.9b
		D	63.9 ± 9.6	40.1 ± 9.5	49.1 ± 11.0	162.0 ± 33.4	23.4 ± 5.8	342.4 ± 35.1	28.7
25	64	T	110.1 ± 19.1c	77.6 ± 17.1c	113.3 ± 26.4c	288.0 ± 68.5c	44.3 ± 9.8c	633.2 ± 67.3c	678.0 ± 67.9c
		D	58.3 ± 10.1	40.1 ± 8.8	55.2 ± 12.9	178.8 ± 42.6	27.1 ± 6.0	359.7 ± 38.1	25.6
30	55	T	70.0 ± 13.2d	48.4 ± 13.5d	63.3 ± 17.5d	214.7 ± 69.2d	27.2 ± 8.2d	422.5 ± 78.2d	457.2 ± 78.2d
		D	51.6 ± 9.7	35.1 ± 9.8	44.0 ± 12.2	178.0 ± 57.4	21.5 ± 7.8	327.5 ± 60.6	29.9
35	36	T	80.8 ± 16.6d	59.3 ± 24.0d	76.0 ± 28.4d	206.0 ± 46.4d	24.0 ± 0.0d	445.5 ± 65.0d	468.3 ± 65.0d
		D	59.6 ± 12.2	43.0 ± 17.4	52.9 ± 19.8	170.8 ± 38.5	19.2 ± 3.3	345.3 ± 50.4	19.7
F			679.23	329.53	293.19	324.74	226.09	1,354.52	1,553.34
df			4, 232	4, 232	4, 232	4, 232	4, 232	4, 232	4, 232
P			<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Developmental times (h) of the same stage are significantly different at five constant temperatures (ANOVA, $P < 0.05$). The developmental times of the same stage followed by the same letter are not significantly different (ANOVA; Tukey's test; $P < 0.05$).

[a] Number of survived adults at each temp.

[b] Included the egg development results.

adult head widths at 15, 30, and 35°C (*t*-test; $P > 0.05$). All sizes of females decreased more rapidly than those of males with increasing temperatures, and this was particularly true of pupal exuvium length and adult body length. Temperature affected all the measured sizes of females more than those of males, and this effect was most profound on adult body length and pupal exuvium length.

Discussion

Temperature is one of the most important factors affecting insect life history, including egg hatching, larval development, emergence, adult longevity, and voltinism. Therefore, temperature-dependent developmental experiments are required not only to understand the general life history of insects but also to use critical rearing information to manage certain indicator insect species. We were able to compare our *G. tokunagai* experiments with previous studies of some chironomid species, which were well known bioassay test species such as *C. riparius*, *C. tentans*, *C. yoshimatsui*, and *C. flaviplumus* Tokunaga.

The number of eggs in a *G. tokunagai* egg mass outnumbered that of *C. riparius* (Downe and Caspary 1973, Watts and Pascoe 2000) but was fewer than that of *C. tentans* (Nolte 1993, Sibley et al. 1997, Watts and Pascoe 2000). Egg and larval development times generally included the time ranges of several chironomid species except those of *G. tokunagai* females at 25°C in this study (Jackson and Sweeney 1995), for example, egg developmental times of *C. tepperi* Skuse (25.9–26.3 h), *C. yoshimatsui* (35–52 h), and *C. flaviplumus* (38–51 h at 25°C) (Kawai and Konishi 1986, Stevens 1998), and larval development times of *C. riparius* (25.4 d in males and 29.3 d in females at 20°C) and *C. tentans* (28.1 d in males and 32.0 d in females at 22°C) (Watts and Pascoe 2000). In general, adult males of many chironomid species emerge earlier than females because females require more time for egg maturation (Oliver 1971, Burlt et al. 1986, Armitage 1995, Stevens 1998, Sildanchandra and Crane 2000, Frouz et al.

Table 5. Developmental time (T) and degree-day (D) for *G. tokunagai* female under five constant temperatures (mean ± SD)

Temperature (°C)	n^a		First instar (h)	Second instar (h)	Third instar (h)	Fourth instar (h)	Pupa (h)	Larva to adult period (h)	Egg to adult period (h)[b]
15	5	T	344.4 ± 34.4a	244.8 ± 39.4a	436.8 ± 93.6a	1,070.4 ± 163.3a	100.8 ± 10.7a	2,197.2 ± 249.1a	2,337.1 ± 249.1a
		D	40.2 ± 4.0	19.4 ± 3.1	49.1 ± 10.5	298.8 ± 45.6	19.7 ± 2.1	421.1 ± 47.7	33.2
20	31	T	199.8 ± 47.7b	134.7 ± 24.5b	202.1 ± 53.2b	729.3 ± 115.2b	57.3 ± 16.0b	1,387.1 ± 142.0b	1,451.7 ± 142.0b
		D	64.9 ± 15.5	38.7 ± 7.1	64.8 ± 17.1	355.5 ± 56.2	23.2 ± 6.5	529.3 ± 56.8	28.7
25	35	T	108.8 ± 13.0c	77.5 ± 20.2c	127.5 ± 34.3c	549.9 ± 86.6c	38.8 ± 11.8c	901.5 ± 73.1c	946.3 ± 73.1c
		D	58.0 ± 6.9	38.4 ± 10.0	67.5 ± 18.2	382.7 ± 60.3	23.1 ± 8.2	548.4 ± 44.5	25.6
30	43	T	71.7 ± 14.2d	48.0 ± 17.5d	80.9 ± 22.8d	361.6 ± 72.5d	25.1 ± 5.2d	585.1 ± 84.7d	619.8 ± 84.7d
		D	53.2 ± 10.6	33.8 ± 12.3	59.6 ± 16.8	326.9 ± 65.6	18.8 ± 7.1	477.8 ± 69.2	29.9
35	27	T	74.4 ± 19.1d	60.4 ± 39.0cd	104 ± 40.5cd	430.2 ± 130.4d	24.9 ± 4.7d	693.1 ± 101.9e	715.9 ± 101.9d
		D	70.7 ± 18.1	55.2 ± 35.6	98.4 ± 38.3	478.6 ± 145.1	24.7 ± 6.9	710.4 ± 104.5	19.7
F			219.85	108.07	116.4	103.22	98.26	458.50	510.50
df			4, 139	4, 139	4, 139	4, 139	4, 139	4, 139	4, 139
P			<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Developmental times (h) of the same stage are significantly different at five constant temperatures (ANOVA, $P < 0.05$). The developmental times of the same stage followed by the same letter are not significantly different (ANOVA; Tukey's test; $P < 0.05$).

[a] Number of survived adults at each temp.

[b] Included the egg development results.

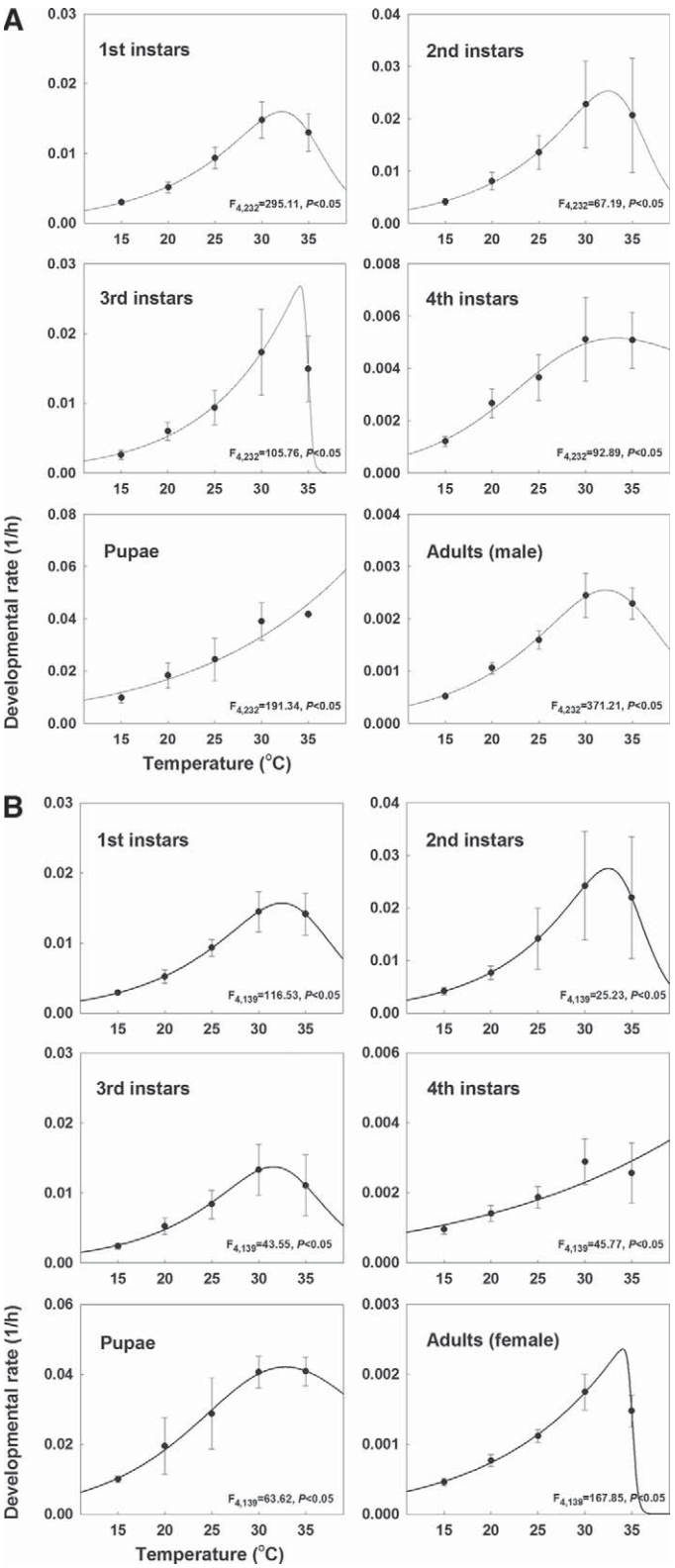

Fig. 4. (A) Developmental rate (1/h) curves for each *G. tokunagai* male developmental stage at five constant temperatures. (B) Developmental rate (1/h) curves for each *G. tokunagai* female developmental stage at five constant temperatures.

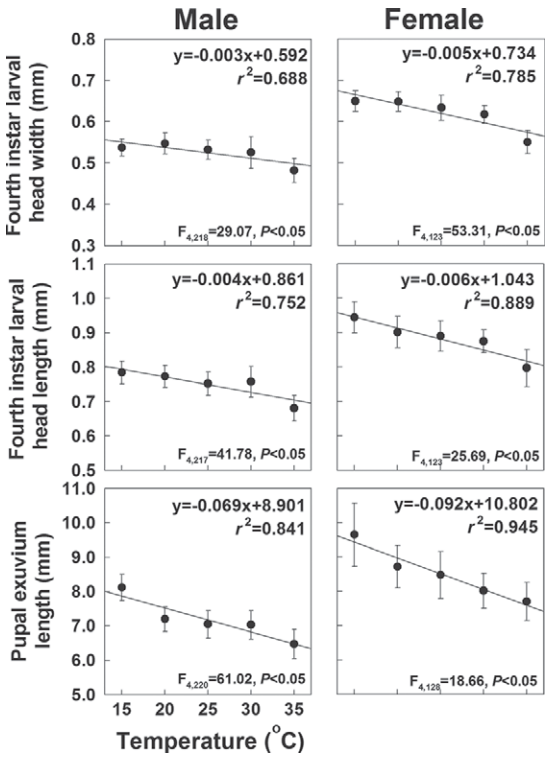

Fig. 5. Larval head width and length and *G. tokunagai* pupal exuvium length (mean ± SD) at five constant temperatures.

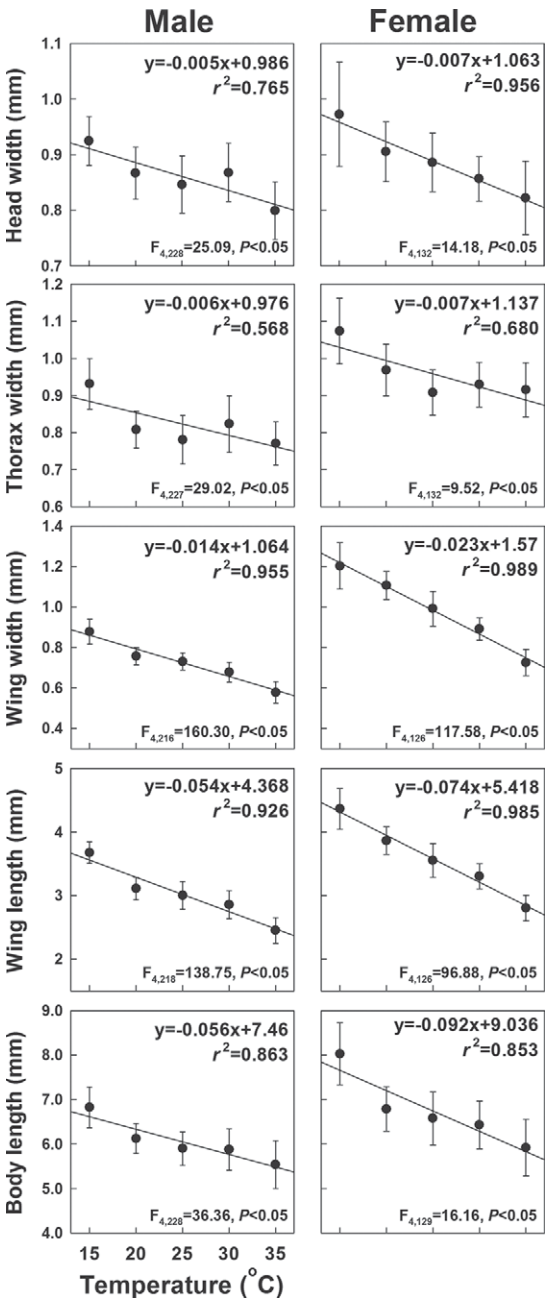

Fig. 6. Adult *G. tokunagai* body sizes (mean ± SD) at five constant temperatures.

2002), but the difference in emerging times between male and female *G. tokunagai* (≈6.7–15.3 d ranging 15–35°C) was larger than that of other species.

The nonlinear development model proposed by Schoolfield et al. (1981) of almost all *G. tokunagai* and *C. crassicaudatus* Malloch life stages (Frouz et al. 2002) certainly fit a bell-shaped curve, as developmental inhibition was observed. The curve peaks of *C. crassicaudatus,* however, were observed at lower temperatures than observed in *G. tokunagai.* The bell-shaped curve was assumed because of the limit in the lack of energy available for larval growth after meeting respiratory energy needs (Frouz et al. 2002). This explains why the most profound high temperature limitation was noted in the fourth instars, wherein the highest proportion of larval growth occurs (Tokeshi 1995).

A lower threshold temperature is important to evaluate the developmental rate in an integrated insect management program (Briere et al. 1999, Wang et al. 2009). The lower threshold temperatures of *G. tokunagai* males and females (11.4 and 10.4°C, respectively) were slightly higher than those determined for *C. tepperi* males and females (10.5 and 10.3°C, respectively) (Stevens 1998), but lower than those of *C. kiinensis* Tokunaga (males and females, 15.6°C) (Surakarn and Yano 1995). This may be the result of thermal adaptation of *G. tokunagai* in the natural habitats, for example, Korean streams, in which temperate monsoonal climate is affected. In arctic lakes, the chironomids *Heterotrissocladius subpilosus* (Kieffer) and *Pseudodiamesa arctica* (Malloch) complete larval development at temperatures <4°C (Oliver 1968).

When compared with the Japanese population of *G. tokunagai* (Yano et al. 1991), the number of eggs, hatching rate, and developmental time and rate were very similar, but the lower threshold temperatures of Japanese *G. tokunagai* (males 13.2°C, females 11.6°C) were somewhat higher than those of our results.

Body size should also be considered to determine the suitable rearing temperature. The body size of *G. tokunagai* was significantly affected by temperature. We measured many of the body parts of larval, pupal, and adult stages, whereas previous chironomid studies included only larval head capsule width, adult wing length, and body length. Our *G. tokunagai* body size measurements closely resembled those of Japanese *G. tokunagai* (head width of fourth instars) (Yano et al. 1991), whereas they were slightly larger than all sizes of *C. riparius*, and smaller than all sizes of *C. tentans* (head capsule width and body length of fourth larval instars) (Watts and Pascoe 2000). Wing length of males was similar to that of *C. flaviplumus* (2.83–3.18 mm) and *C. yoshimatsui* (2.91–3.53 mm) (Kawai and Konishi 1986). All measured sizes of *G. tokunagai* females were larger than those of males. It is known that the size at which a larva is physiologically mature and capable of pupating is also affected by gender (Oliver 1971).

Temperature induced body sizes differences in many ectotherms may simply be a consequence of the effect of temperature on cellular development (Van Voorhies 1996). Numerous experiments have shown that ectotherms grow larger at lower temperatures (Van Voorhies 1996), and chironomid larvae that develop at high temperature also are smaller in size (Konstantinov 1958, Oliver 1971). The head width of the fourth instar larvae and wing length of *C. crassicaudatus* is smaller at higher temperatures, and those of the females are significantly larger than those of males (Frouz et al. 2002). The wing length of *C. kiiensis* males significantly shortens with increasing temperatures (Surakarn and Yano 1995). Our results also showed that all *G. tokunagai* life stages tended to decrease in size with increases in temperature from 15 to 35°C.

Our results demonstrated that *G. tokunagai* is a potential test insect species if maintained in a laboratory under optimal temperature and rearing conditions. Our experiments showed that *G. tokunagai* not only maintained relatively high hatching, emergence rates, and body sizes under laboratory conditions but also tolerated relatively high temperatures. This experimental population of *G. tokunagai* also has been successfully reared for >30 generations for 4 yr in the laboratory (Baek et al. 2011). Therefore, *G. tokunagai* could be considered for various environmental studies as test organisms, because this species fulfilled the selection criteria of test organisms (Buikema et al. 1982). From the results of this study, the approximate rearing temperature for *G. tokunagai* is suggested to be 25–30°C for egg hatching rate (>97.3%), development time (19–40d), and emergence rate (>79.5%).

Acknowledgments

This research was supported by the "Eco-Innovation" project of KEITI under the Ministry of Environment of Korea (R1104911 [2011]).

References Cited

Armitage, P. D., P. S. Cranston, and L.C.V. Pinder. 1995. The Chironomidae. Chapman & Hall, London.

Baek, M. J., T. J. Yoon, J. M. Hwang, H. J. Kang, S. J. Lee, and Y. J. Bae. 2011. Four-year successive rearing of *Glyptotendipes tokunagai* Sasa (Diptera: Chironomidae) under laboratory conditions. Entomol. Res. 42: 276.

Briere, J. F., P. Pracros, A. Y. Roux, and J. S. Pierre. 1999. A novel model of temperature-dependent development for arthropods. Environ. Entomol. 28: 22–29.

Buikema, A. L. Jr., B. R. Niederlehner, and J. Jr. Cairns. 1982. Biological monitoring partIV-toxicity testing. Water Res. 16: 239–262.

Burlt, E. T., R.G.O. Perry, and A. J. McLachlan. 1986. Feeding and sexual dimorphism in adult midges (Diptera: Chironomidae). Holarct. Ecol. 9: 27–32.

Downe, A.E.R., and V. G. Caspary. 1973. The swarming behaviour of *Chironomus riparius* (Diptera: Chironomidae) in the laboratory. Can. Entomol. 105: 165–171.

Frouz, J., A. Ali, and R. J. Lobinske. 2002. Influence of temperature on developmental rate, wing length, and larval head capsule size of pestiferous midge *Chironomus crassicaudatus* (Diptera: Chironomidae). J. Econ. Entomol. 95: 699–705.

Jackson, J. K., and B. W. Sweeney. 1995. Egg and larval development times for 35 species of tropical stream insects from Costa Rica. J. N. Am. Benthol. Soc. 14: 115–130.

Kawai, K., and K. Konishi. 1986. Fundamental studies on chironomid allergy: I. Culture methods of some Japanese chironomids (Chironomidae, Diptera). Jpn. H. Sanit. Zool. 37: 47–57.

Konstantinov, A. S. 1958. On the growth type of the midges of the family Chironomidae. Dokl. Akad. Nauki SSSR 120: 1151–1154.

Lee, J. H., S. H. Lee, Y. J. Bae, K. J. Cho, K. S. Ryoo, Y. Kim, and M. P. Jung. 2009. Assessment techniques for ecological effects on heavy metal pollution using invertebrate biomarkers. Jeonghaengsa, Seoul, Korea.

Nolte, U. 1993. Egg masses of Chironomidae (Diptera). A review, including new observations and a preliminary key. Entomol. Scand. Suppl. 43: 1–75.

OECD. 2001. Guideline for the testing of chemicals, sediment-water chironomid toxicity test using spiked sediment. Guideline 218. Organization for Economic Cooperation and Development, Paris, France.

Oliver, D. R. 1968. Adaptations of arctic Chironomidae. Ann. Zool. Fenn. 5: 111–118.

Oliver, D. R. 1971. Life history of the Chironomidae. Annu. Rev. Entomol. 16: 211–230.

SAS Institute. 1999. SAS version 8.1. SAS Institute, Cary, NC.

Sasa, M. 1989. Chironomidae of Japan: checklist of species recorded, key to males and taxonomic note. Res. Rep. NIES 125: 1–177.

Schoolfield, R. M., P.J.H. Sharp, and C. E. Magnuson. 1981. Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. J. Theor. Biol. 88: 719–731.

Sharp, P.J.H., and D. W. DeMichele. 1977. Reaction kinetics of poikilotherm development. J. Theor. Biol. 64: 649–670.

Sibley, P. K., D. A. Benoit, and G. T. Ankley. 1997. The significance of growth in *Chironomus tentans* sediment toxicity tests. Relationship to reproduction and demographic end points. Environ. Toxicol. Chem. 16: 336–345.

Sildanchandra, W., and M. Crane. 2000. Influence of sexual dimorphism in *Chironomus riparius* Meigen on toxic effects of cadmium. Environ. Toxicol. Chem. 19: 2309–2313.

Stevens, M. M. 1998. Development and survival of *Chironomus tepperi* Skuse (Diptera: Chironomidae) at a range of constant temperatures. Aquat. Insect 20: 181–188.

Surakarn, R., and K. Yano. 1995. Development of a paddy-dwelling chironomid, *Chironomus kiiensis* (Diptera, Chironomidae) under different temperatures. Jpn. J. Entomol. 63: 389–398.

Sweeney, B. W. 1984. Factors influencing life-history patterns of aquatic insects, pp. 56–100. *In* V. H. Resh and D. M. Rosengerg (eds.), The ecology of aquatic insects. Praeger, New York.

Tokeshi, M. 1995. Life cycles and development, pp. 224–268. *In* P. D. Armitage, P. S. Cranston, and L.C.V. Pinder (eds.), The Chironomidae: the biology and ecology of non-biting midges. Chapman & Hall. London, United Kingdom.

US EPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-94/024, Technical Report, Washington, DC.

Wagner, T. L., H. Wu, P.J.H. Sharpe, R. M. Schoolfield, and R. N. Coulson. 1984. Modeling insect development rates: a literature review and application of a biophysical model. Ann. Entomol. Soc. Am. 77: 208–225.

Wang, J. J., J. H. Tsai, Z. M. Zhao, and L. S. Li. 2000. Development and reproduction of the psocid *Liposcelis bostrychophila* (Psocoptera: Liposcelididae) as a function of temperature. Ann. Entomol. Soc. Am. 93: 261–270.

Watts, M. M., and D. Pascoe. 2000. A comparative study of *Chironomus riparius* Meigen and *Chironomus tentans* Fabricius (Diptera:Chironomidae) in aquatic toxicity tests. Arch. Environ. Contam. Toxicol. 39: 299–306.

Van Voorhies, W. A. 1996. Bergmann size clines: a simple explanation for their occurrence in ectotherms. Evolution 50: 1259–1264.

Weltje, L., H. Rufli, F. Heimbach, J. Wheeler, M. Vervliet-Scheebaum, and M, Hamer. The chironomid acute toxicity test: development of a new test system. Integr. Environ. Assess. Manag. 6: 301–307.

Yano, K., M. Takayama, and T. Ueda. 1991. Biology of *Glyptotendipes tokunagai* Sasa (Diptera: Chironomidae) 1. Development of immature stages. Trans. Shikoku Entomol. Soc. 19: 177–188.

Received 7 November 2011; accepted 21 March 2012.