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2 **Effects of temperature on life histories of three endangered**

3 **Japanese diving beetle species**

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12 **Running title:** *Life histories of three diving beetle species*

13

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18

1 **Abstract**

2 To elucidate population-increasing factors in the diving beetle, *Cybister tripunctatus lateralis*
3 (Fabricius) (Coleoptera: Dytiscidae), in Japan in recent years, life histories and oviposition
4 patterns were compared among three endangered diving beetle species, *Cybister brevis* Aubé
5 (qualified by the Japanese Red Data List as ‘near threatened’), *Cybister chinensis*
6 Motschulsky (vulnerable), and *C. tripunctatus lateralis* (vulnerable). Oviposition in *C. brevis*,
7 *C. chinensis*, and *C. tripunctatus lateralis* was observed from late April to mid-June, from late
8 April to early July, and from late May to mid-August, respectively, under semi-outdoor
9 conditions. There were no interspecies differences in total hatchling production during the
10 reproductive season. In rearing experiments at various temperatures (20, 23, 25, 28, and
11 30 °C), the mortality of *C. tripunctatus lateralis* larvae was higher at 20 °C, and gradually
12 lower with increasing temperature up to 30 °C. Adult body size of females in *C. tripunctatus*
13 *lateralis* is larger than that of males but there were no significant differences among
14 temperatures (25–30 °C). *Cybister brevis* had a higher emergence rate at 23–28 °C than at 20
15 and 30 °C. In *C. brevis* the body size of adults reared at 25 or 28 °C was significantly larger
16 than at other temperatures. *Cybister chinensis* did not differ in emergence rate and adult body
17 size among the five temperature conditions. The developmental zero (i.e., the lower
18 developmental threshold) from the first instar to adult emergence was 11.1 °C for *C. brevis*,
19 8.7 °C for *C. chinensis*, and 16.8 °C for *C. tripunctatus lateralis*. We speculate how the
20 influence of global warming may have a positive impact on the growth and survival of *C.*
21 *tripunctatus lateralis*.

22

23 **Abbreviated abstract (2-3 sentences, max. 80 words)**

24 To elucidate population-increasing factors in the diving beetle *Cybister tripunctatus lateralis*
25 (Coleoptera: Dytiscidae), in Japan in recent years, life histories were compared among three
26 endangered diving beetle species, *Cybister brevis*, *C. chinensis*, and *C. tripunctatus lateralis*.
27 Various life-history parameters were determined in rearing experiments under a temperature
28 range from 20 to 30 °C. Our results suggest that the influence of global warming may have a
29 positive effect on the increasing of *C. tripunctatus lateralis*. [74 words]

30

31 **Graphic for Table of Contents**

32 Figure 4

33

1 **Introduction**

2 Predacious diving beetles (Coleoptera: Dytiscidae) are among the most fearsome predators in
3 freshwater environments (Yee, 2014). They are important predators and are often at the upper
4 end of the food web in aquatic communities in fishless ponds (Cobbaert et al., 2010; Culler et
5 al., 2014). Almost half of Japanese diving beetles (approximately 46%) inhabit paddy field
6 water systems, which are composed of paddy fields, agricultural ditches, and irrigation ponds
7 (Saijo, 2001; Mori & Kitayama, 2002; Nakajima et al., 2020). The diversity of diving beetles
8 is declining in paddy field water systems, due to farmland consolidation, increasing paddy
9 field abandonment, and the use of pesticides and herbicides (Nishihara et al., 2006).
10 Approximately 45% of diving beetles inhabiting paddy field water systems are listed on the
11 Red List of Japan (Ministry of the Environment of Japan, 2019).

12 There are seven species of the genus *Cybister* from Japan, large beetles exceeding
13 20 mm in body length, six of which are listed in the Japanese Red Data List due to their
14 annually decreasing population size (Ministry of the Environment of Japan, 2019). In this
15 study, we focused on *Cybister tripunctatus lateralis* (Fabricius), *Cybister brevis* Aubé, and
16 *Cybister chinensis* Motschulsky (synonym: *Cybister japonicus* Sharp), which are widely
17 distributed in Japan (Figure 1). *Cybister tripunctatus lateralis* (20–29 mm in body length) is
18 distributed in the southern and southwestern islands of Honshu, the southern part of China,
19 and eastern Europe (Figure 1). This species is designated as ‘vulnerable’ (VU) in the Japanese
20 Red Data List, being extinct in eight prefectures (Association of Wildlife Research &
21 EnVision, 2019). *Cybister brevis* (20–25 mm in body length) is distributed in China, Taiwan,
22 the Korean Peninsula, and Japan excluding Hokkaido and the Ryukyu Islands (Mori &
23 Kitayama, 2002). This species is designated as ‘near threatened’ (NT) in the Japanese Red
24 Data List, being extinct in Tokyo (Association of Wildlife Research & EnVision, 2019).
25 *Cybister chinensis*, the largest species of Japanese diving beetles (33–42 mm in body length),
26 is distributed in the Korean Peninsula, Taiwan (?), China, Siberia, and Japan excluding the
27 Ryukyu Islands (Mori & Kitayama, 2002). This species is designated as a VU species in the
28 Japanese Red Data List, being extinct in five prefectures.

29 Interestingly, in recent years, there has been an increase in new distributional and
30 rediscovery records of *C. tripunctatus lateralis*, mainly in western Japan (Shimono, 2015;
31 Mitamura et al., 2017; Nakajima et al., 2020). Before 2010, *C. tripunctatus lateralis* only
32 remained in southern regions, such as western Shikoku, southern Kyushu, and the Ryukyu
33 Islands in Japan (Nishihara et al., 2006); however, in 2012, it was first discovered in Ishikawa

1 Prefecture (Shimada & Tomisawa, 2014). Therefore, *C. tripunctatus lateralis* was
2 exceptionally evaluated to have increased its population in contrast to other large-bodied
3 *Cybister* diving beetle species; the Japanese Red Data List by the Ministry of the Environment
4 changed its status in 2012 from CR to VU (Ministry of the Environment of Japan, 2015). We
5 speculate that *C. tripunctatus* is positively affected by increased temperatures, such as those
6 due to global warming. The spread of diving beetles due to such warming has also been
7 reported in the European beetles *Cybister lateralimarginalis* (De Geer) (Thomas, 2009),
8 *Rhantus suturalis* (Macleay) (Bilton, 2014), and *Eretes sticticus* (L.) (Hajek et al., 2014);
9 however, no studies have compared the life histories of closely related species.

10 We hypothesized that increased temperature contributes to increase in population size
11 of *C. tripunctatus lateralis* (southern species) in Japan. Therefore, our predictions are: (1) the
12 beginning of oviposition in *C. tripunctatus lateralis* is later than in the other two species, (2)
13 larval survival increases with increasing rearing temperature in *C. tripunctatus lateralis*, and
14 (3) developmental zero (i.e., the lower developmental threshold) in *C. tripunctatus* is highest
15 of the three species.

16

17 **Materials and methods**

18 **Oviposition pattern**

19 To compare the oviposition patterns, seven pairs (male and female) of *C. chinensis* and six
20 pairs of *C. brevis* and *C. tripunctatus lateralis* were collected from ponds in Kumamoto
21 Prefecture, Kyushu, in early April 2015 (Figure 1). To observe the oviposition pattern, a male
22 and a female were introduced into an aquarium (25 cm long, 19 cm wide, 20 cm high for *C.*
23 *brevis* and *C. tripunctatus lateralis* and 35 × 22 × 26 cm for *C. chinensis*). We used a larger
24 aquarium for *C. chinensis* because of its larger body. In the aquarium, gravel and driftwood,
25 and water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae), were used as
26 perching site and egg-laying materials, respectively. Depending on their size, two (if 10–15
27 cm in diameter) or one (16–25 cm in diameter) *E. crassipes* individuals were introduced into
28 each aquarium.

29 To keep the tanks at the same temperature and natural daylength as the outdoors, the
30 aquaria were set up in the laboratory (Faculty of Education, Nagasaki University), which were
31 screened and open air was introduced. Fluorescent lights or light-emitting diodes (LED) and
32 tropical business lights (CLEAR LED600; Gex, Osaka, Japan) were installed at the top of the
33 aquarium to shine strong light during the natural photoperiod. The lights were turned on from

1 06:30 to 18:00 h in April–June, and from 06:00 to 18:30 h in July–September. For the adult
2 food, 2-3 dried shrimp for tropical fish (Spectrum Brands, Tokyo, Japan) were fed per pair to
3 *C. brevis* and *C. tripunctatus lateralis*, and 3-4 were fed to *C. chinensis* every 2 days. Because
4 the larva takes about 10-20 days to hatch, the *E. crassipes* was replaced every 10-13 days. The
5 collected *E. crassipes* was introduced into a tub (23 cm diameter, 9 cm high), and
6 dechlorinated water was poured into the tub until the oviposition hole made by the female
7 beetle was submerged in water. The number of hatched larvae between 24 April and 26
8 September 2015 was recorded daily. Observation of *C. brevis* and *C. chinensis* females ceased
9 on 26 August because no hatching larvae were found for 1 month after 26 July. Similarly,
10 observation of *C. tripunctatus lateralis* females ceased on 26 September because no hatching
11 larvae were found after 2 September.

12 To compare the number of laid eggs among the three species, the total number of eggs laid
13 during the observation period of each female was calculated, and the non-parametric Kruskal–
14 Wallis rank sum test was performed using R software (R Core Team, 2016).

15

16 **Effect of temperature on immature development**

17 ***Egg***

18 To obtain and examine the developmental zero (i.e., the lower developmental threshold)
19 during the egg period and the effective accumulated temperature, multiple male and female
20 adults were introduced into the same aquarium, and eggs were freely laid into water hyacinth
21 from June to July 2014, April to July 2015, and June to July 2016. We used a large aquarium
22 (60 cm long, 30 cm wide, 35 cm high) for 17 females and six males of *C. chinensis*. For *C.*
23 *brevis* and *C. tripunctatus lateralis*, we used three small aquaria (33 cm long, 16 cm wide, 22
24 cm high): eight pairs of *C. brevis*, nine pairs of *C. brevis*, and six females and seven males of
25 *C. tripunctatus lateralis* were introduced into these aquaria. The aquaria were kept under the
26 abovementioned semi-natural conditions. Water hyacinth was used as the egg-laying material.
27 The water hyacinth was checked every day to determine whether there were any oviposition
28 holes made by females. Water hyacinth with new oviposition holes on the surface were
29 transferred into a plastic cup (129 mm diameter, 97 mm high) filled with dechlorinated water.
30 The plastic cups were placed at 20, 23, 25, 28, or 30 °C, and checked at 24-h intervals for
31 newly hatched larvae. Each water hyacinth was used once only.

32 ANOVA was performed with species and temperature as independent variables and
33 egg period (days) as response variable. If the temperature and species effects were significant,

1 multiple comparisons among species were performed at each temperature using the Tukey–
2 Kramer test in R (R Core Team, 2016).

3

4 ***Larva to adult***

5 The hatched larvae were individually transferred to plastic cups (129 mm diameter, 60 mm
6 high, 30 mm water depth, or 101 mm diameter, 45 mm high, 15 mm water depth) (see Table
7 S1) and maintained at 20, 23, 25, 28, or 30 °C to determine the developmental zero and
8 effective cumulative temperature. A plastic net (12 cm long, 3 cm wide; 3 mm mesh) was
9 used as a perch for larvae. As a feed, a frozen cricket, *Gryllus bimaculatus* De Geer
10 (Orthoptera: Gryllidae) (Tsukiyono Farm, Gunma, Japan), was given to each larva, and the
11 size of the frozen cricket was determined according to the instar of the larva (see Table S2).
12 We used frozen crickets because the larvae of all three species are known as insectivorous
13 (Ohba, 2009a,b; Ohba & Inatani, 2012). We assumed that the frozen crickets are uniform in
14 nutritional values among daily food supply. We checked the larval instar (moulting or not) and
15 changed the frozen cricket and dechlorinated tap water every day (almost every 24 h). Similar
16 to Ohba (2009a,b), third instars that did not eat the prey within 1 h after it was provided were
17 moved to a cup (129 mm diameter, 97 mm high) filled with wet peat moss for pupation. The
18 day that third instars burrowed into the peat moss was recorded as the last day of the larval
19 period. New adults emerging from the peat moss were measured for their maximum abdomen
20 width using callipers as an accurate body size index.

21 To examine the effect of temperature on the developmental period, we applied a
22 generalised linear model (GLM) using R (R Core Team, 2016) with a binomial distribution,
23 which incorporated the temperature and species as the explanatory variables and the adult
24 emergence rate as the response variable. If the interaction was significant, χ^2 tests compared
25 the temperature within species. The overall significance level was adjusted using the
26 sequential Bonferroni method (Rice, 1989).

27 To compare the development period among species, data from 23 to 30 °C were
28 analysed because most individuals of *C. tripunctatus lateralis* under 20 °C conditions died
29 before adult emergence (see Results); there was only one sample of *C. tripunctatus lateralis* at
30 20 °C, which was excluded from the analysis. Multiple comparisons among species were
31 performed at each stage using the Tukey–Kramer test in R (R Core Team, 2016).

32 Two-way ANOVA was performed to compare the body width of adults at each
33 temperature by sex and development temperature. Because *C. brevis* and *C. chinensis* did not

1 show any significant difference between males and females (see Results), the differences
2 among temperature were examined for each species after the sex data were pooled. However,
3 sex differences were observed for *C. tripunctatus lateralis*, so we did not pool the sex data for
4 this species.

5

6 **Estimation of developmental zero and annual generations**

7 Linear regression of temperature (T) and developmental rate (1/D) were used to estimate the
8 lower threshold temperature for development and the thermal constant for each species. The
9 relationship between 1/D and T is described by the regression equation $1/D = a + bT$ and $K =$
10 $(T - T_0)D$, where D is developmental period, T is temperature, a is the intercept of the
11 regression line, b is a coefficient, T_0 is developmental zero, and K is the effective accumulated
12 temperature. The lower thresholds were calculated by solving the equation for T when $1/D =$
13 0. The thermal constants required for the completion of development for each of the stages
14 were determined using $K = 1/b$. In this study, the development rate at 30 °C for the three
15 species was excluded from the linear regression because heat stress usually begins between 28
16 and 32 °C in most insect taxa (Kiritani, 2012). Actually, if data at 30 °C were used for the
17 regression analysis for all three species, the R^2 value decreased in most stages (see Table S3),
18 so data at 20, 23, 25, and 28 °C were used in this study. As the survival rate at 20 °C was low
19 and the number of samples was 1, the data of 23, 25, and 28 °C were used for *C. tripunctatus*.
20 Therefore, the R^2 value from the first instar to the emergence period was close to 1.0 for all
21 three species.

22 The annual generation numbers of each species in their habitat were estimated
23 using developmental zero, the accumulated effective temperature estimated by this study, and
24 the mean temperatures provided by the Automated Meteorological Data Acquisition System,
25 Kumamoto City in 2015–2019. In this study, we estimated the egg period and hatching date,
26 and the next day was regarded as the first larval appearance.

27 For all statistical tests the significance threshold was set at $\alpha = 0.05$, adjusted using
28 the sequential Bonferroni method, if applicable.

29

30 **Results**

31 **Oviposition pattern**

32 During the entire observation period, in total 70 hatched larvae were confirmed from all six
33 pairs of *C. tripunctatus lateralis*. Sixty-nine larvae were found in five out of the six pairs of *C.*

1 *brevis*. Forty-nine larvae hatched from four of the seven pairs in *C. chinensis* (Table S4). No
2 difference was observed among the three species (Kruskal–Wallis rank sum test: $P = 0.75$).

3 Females of the three species began to lay eggs from late April (Figure 2). The
4 oviposition was consecutively confirmed until mid-June in *C. brevis* and early July in *C.*
5 *chinensis*. However, oviposition in *C. tripunctatus lateralis* females was not confirmed
6 between 6 and 25 May, and five of six females started to lay eggs from late May to mid-
7 August (Figure 2, Table S4).

8

9 **Effect of temperature on immature development**

10 ***Egg***

11 Species and temperature affected the duration of the egg period, but not their interaction (two-
12 way ANOVA, species: $F_{2,228} = 33.9$; temperature: $F_{1,228} = 367.0$, both $P < 0.001$;
13 species*temperature: $F_{2,228} = 1.98$, $P = 0.14$). At 20 and 23 °C, *C. tripunctatus lateralis* had
14 the shortest egg period of the three species (Tukey–Kramer test: $P < 0.05$), but at 25, 28, and
15 30 °C, there was no difference among the species (Figure 3).

16

17 ***Survival rate***

18 – The effect of temperature on the survival rate was different among species (Table 1). The
19 survival rate of *C. tripunctatus lateralis* was highest at 30 °C, extremely low at 20 °C, and
20 intermediate at the three intermediate temperatures (Figure 4). The survival rate of *C. brevis*
21 was high at 23–28 °C, but decreased at 20 and 30 °C. In *C. chinensis*, temperature did not
22 affect survival rate (Figure 4).

23

24 ***Larval and pupal period***

25 At 20 °C, the first and second instar in *C. brevis* was significantly longer than that in *C.*
26 *chinensis* (Table 2). The third instar was significantly longer in *C. chinensis* than in *C. brevis*.
27 However, no significant differences were observed among species in the pupae and the first-
28 instar to adult (total juvenile periods) periods.

29 At 23 °C, the first-instar period was the longest in *C. tripunctatus lateralis*, followed
30 by *C. brevis* and *C. chinensis* in that order. The second-instar period was longer in *C.*
31 *tripunctatus lateralis* than in *C. chinensis*; however, the third-instar period was shorter in *C.*
32 *tripunctatus lateralis* than in *C. chinensis*. The pupal period did not differ among the three
33 species. The period from the total juvenile periods was significantly longer for *C. tripunctatus*

1 *lateralis* than for the other two species.

2 At 25 °C, the first and second instars did not differ among the three species, but the
3 third instar of *C. chinensis* was significantly longer than that of the other two species. The
4 pupal and total juvenile period in *C. tripunctatus lateralis* were longer than those in the other
5 two species.

6 At 28 °C, the first and second-instar periods in *C. brevis* were significantly longer
7 than in the other two species; however, the third-instar period in *C. tripunctatus lateralis* was
8 significantly shorter. There were no significant differences among the three species during the
9 pupal period and the total juvenile period.

10 At 30 °C, the third-instar period in *C. chinensis* was significantly longer than that in
11 the other two species. There were no significant differences among the three species in the
12 second, pupal, and total juvenile periods.

13 Adult body size of *C. tripunctatus lateralis* was affected by sex ($F_{1,74} = 4.9$, $P =$
14 0.031), but not by temperature ($F_{2,74} = 2.01$, $P = 0.14$) or their interaction ($F_{2,74} = 0.23$, $P =$
15 0.80) (Figure 5). Overall, female *C. tripunctatus lateralis* were larger than males. Adult body
16 size of *C. brevis* was affected by temperature ($F_{1,65} = 8.6$, $P = 0.004$), but not by sex ($F_{1,65} =$
17 1.74 , $P = 0.19$) or their interaction ($F_{1,65} = 2.26$, $P = 0.14$). Adults reared under 25 and 28 °C
18 were significantly larger than those at other temperatures. Adult body size of *C. chinensis* was
19 unaffected by sex or temperature ($P > 0.25$).

20

21 **Estimation of developmental zero and annual generations**

22 The third-instar period showed the lowest developmental zero at all stages in all three
23 species (Table 3). The developmental zero for all stages was in the range of 3.5–13.8 °C for *C.*
24 *chinensis*, 3.8–13.5 °C for *C. brevis*, and 8.2–19.6 °C for *C. tripunctatus lateralis*. Excluding
25 the egg stage, the developmental zero of *C. tripunctatus* was higher than that of the other two
26 species (Table 3). Using the developmental zero and accumulated effective temperature, the
27 annual generation numbers in Kumamoto City were estimated at 2.2 for *C. tripunctatus*
28 *lateralis*, 2.83 for *C. brevis*, and 2.68 for *C. chinensis*.

29

30 **Discussion**

31 In line with our first prediction, egg laying started at different periods among the three
32 species. *Cybister brevis* and *C. chinensis* started laying eggs in late April, whereas almost all
33 *C. tripunctatus lateralis* started 1 month later, in late May (Table S4). However, the numbers

1 of eggs laid in our result may be different from the natural situation. In this study, water
2 hyacinth which is an invasive plant, was used under constant conditions. The numbers of egg
3 laid in our study might be over- or underestimated. The reduction in appropriate oviposition
4 plants is thought to be one of the factors reducing *C. chinensis* (Ichikawa, 2002), and may be
5 responsible for the decline in the population of all three species. Elucidating the preferred
6 oviposition plants of each species will be important in future research, as in the study of
7 *Dytiscus sharpi* Wehncke (Inoda, 2011a,b). In addition to a later start, *C. tripunctatus lateralis*
8 finished their egg period earlier than did the other two species at low temperatures (20 and
9 23 °C). The larvae of *C. tripunctatus lateralis* may emerge earlier than those of the other two
10 species, even if oviposition begins later. In this case, interactions among the three species are
11 expected to occur; therefore, it is necessary to investigate larval occurrence in the field in the
12 future.

13 In line with our second prediction, the survival rate of *C. tripunctatus lateralis*
14 increased at 30 °C, at 20 °C, most individuals died. Meanwhile, *Cybister brevis* showed the
15 highest survival rates at 23 and 28 °C rather than at 30 °C, indicating less resistance to high
16 temperatures. In addition, a comparison of adult body sizes among temperatures showed that
17 *C. brevis* became larger at 25–30 °C. *Cybister tripunctatus lateralis* also had such a tendency,
18 but the number of low-temperature (20 and 23 °C) samples was small and statistical analysis
19 could not be performed here. In contrast, *C. chinensis* did not show significant differences in
20 survival rate and adult body size, which may be a characteristic of this species. The results of
21 the comparison among temperatures concerning survival rates and body size will reflect their
22 distribution.

23 In line with our third prediction, the developmental zero was the highest in *C.*
24 *tripunctatus lateralis*, followed by *C. brevis* and *C. chinensis*. Therefore, the growth of *C.*
25 *tripunctatus lateralis* requires a higher temperature environment to complete their
26 development than do the other two species. In addition, *C. tripunctatus lateralis* larval
27 development of each stage at the low temperatures (23 and 25 °C) was slower than that of the
28 other two species. Kiritani (2012) examined the developmental zero of various insects and
29 reported that the average developmental zero of 31 species of the order Coleoptera was
30 10.9 °C. In our results, the developmental zero of *C. tripunctatus lateralis* was 16.8 °C, and
31 was highest at the second instar (19.6 °C). As the accumulated effective temperature of *C.*
32 *tripunctatus lateralis* was the lowest among the three species, it is expected that the number of
33 generations can be increased more easily in warm regions in this species than in the other two

1 species.

2 We estimated the annual generation numbers in Kumamoto City to be 2.2 for *C.*
3 *tripunctatus lateralis*, 2.8 for *C. brevis*, and 2.7 for *C. chinensis*. Note, however, that we did
4 not include pre-oviposition periods in this estimation. Therefore, the actual annual generation
5 numbers for each species may be lower than 2. It is necessary to investigate the seasonal
6 prevalence of each species to determine the annual number of generations in the field survey.

7 In conclusion, we speculate that the influence of global warming may have a
8 positive effect on the growth and survival of *C. tripunctatus lateralis*, which may have
9 resulted in the recent increase in this species in western Japan. The three species are sympatric
10 in some areas and have overlapping larval feeding habits (Ohba, 2009a,b; Ohba & Inatani,
11 2012); thus, overlapping larval emergences may lead to interspecific competition. Therefore,
12 it is necessary to investigate the behavioural interaction of adults from the viewpoint of
13 interspecific competition for long-term conservation actions.

14

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23

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1 **Figure captions**

2 **Figure 1** Distribution of the three *Cybister* diving beetle species. The stars mark Kumamoto
3 Prefecture, where we collected beetles for this study.

4
5 **Figure 2** Oviposition patterns (mean + SE no. hatched larvae) of three *Cybister* species. The
6 total number of hatched larvae was not significantly different among species (Tukey–Kramer
7 test: n.s., $P>0.05$).

8
9 **Figure 3** Comparison of egg periods (days) among *Cybister tripunctatus lateralis* (Ct, dark
10 grey), *C. brevis* (Cb, light grey), and *C. chinensis* (Cc, white) under constant temperature
11 conditions (20–30 °C). Different letters near the boxplots within a temperature denote
12 statistical differences in mean egg period among species (Tukey–Kramer test: $P>0.05$). The
13 thick line, the upper and lower boxes, the whiskers, the dots shows the median, upper and
14 lower quartile, the range between minimum and maximum and outliers, respectively. Figures
15 just above the horizontal axis indicate sample size.

16
17 **Figure 4** Comparison of survival rates of the three *Cybister* species among five constant
18 temperatures. Survival rates within a species capped with different letters are significantly
19 different (χ^2 test with sequential Bonferroni correction: $P<0.05$; n.s., no significant
20 differences). The numbers within the bars indicate initial sample sizes.

21
22 **Figure 5** Comparison of adult body width (mm) of the three *Cybister* species among five
23 constant temperatures. Different letters near the boxplots within a species denote statistical
24 differences in mean body width (Tukey–Kramer test: $P<0.05$; n.s., no significant differences).
25 Data from 20 and 23 °C in *C. tripunctatus lateralis* were excluded from the analysis due to
26 the small sample sizes. The numbers just above the horizontal axes indicate sample sizes. F,
27 female; M, male. The thick line, the upper and lower boxes, the whiskers, the dots shows the
28 median, upper and lower quartile, the range between minimum and maximum and outliers,
29 respectively.

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31

1 **Table 1** Results from the generalized linear model (GLM) analyzing the effect of temperature
 2 on the emergence rate of *Cybister* species

	Estimate	SE	Z	P
(Intercept)	-8.35	1.420	-5.88	<0.00001
vs. <i>C. brevis</i> (Cb) ^a	7.45	1.689	4.409	0.00001
vs. <i>C. chinensis</i> (Cc) ^b	9.69	2.197	4.412	0.00001
Temperature (T)	0.33	0.054	6.083	<0.00001
Cb:T	-0.28	0.065	-4.356	0.00001
Cc:T	-0.34	0.086	-4.008	0.00006

3 ^aThe coefficient indicates the relative effect of *C. brevis* compared with *C. tripunctatus*
 4 *lateralis* at 20 °C.

5 ^bThe coefficient indicates the relative effect of *C. chinensis* compared with *C. tripunctatus*
 6 *lateralis* at 20 °C.

1 **Table 2** Mean (\pm SE) developmental period (days) of each stage of the three *Cybister* species

Temperature (°C)	Species	n	1st instar	2nd instar	3rd instar	Pupa	1st instar-adult
20	<i>C. tripunctatus lateralis</i>	1	12.0	15.0	13.0	56.0	96.0
	<i>C. brevis</i>	9	20.1 \pm 1.45a	16.3 \pm 0.93a	15.0 \pm 1.46a	58.6 \pm 5.35a	110.0 \pm 7.30a
	<i>C. chinensis</i>	13	12.2 \pm 0.45b	13.1 \pm 0.52b	18.2 \pm 0.63b	55.6 \pm 2.24a	99.1 \pm 2.62a
23	<i>C. tripunctatus lateralis</i>	5	16.8 \pm 0.20a	17.0 \pm 1.14a	10.8 \pm 0.80a	51.4 \pm 4.39a	96.0 \pm 4.47a
	<i>C. brevis</i>	8	12.0 \pm 0.78b	14.3 \pm 1.00a	13.5 \pm 1.15ab	41.3 \pm 4.78a	81.0 \pm 4.16b
	<i>C. chinensis</i>	15	8.9 \pm 0.48c	10.3 \pm 0.21b	15.2 \pm 0.39b	42.7 \pm 1.07a	77.1 \pm 1.09b
25	<i>C. tripunctatus lateralis</i>	18	9.7 \pm 0.80a	9.1 \pm 0.25a	10.0 \pm 0.30a	47.1 \pm 2.87a	75.8 \pm 3.12a
	<i>C. brevis</i>	19	9.2 \pm 0.25a	9.1 \pm 0.44a	11.1 \pm 0.33a	33.1 \pm 1.71b	62.5 \pm 1.90b
	<i>C. chinensis</i>	19	7.9 \pm 0.37a	8.2 \pm 0.34a	13.6 \pm 0.38b	33.7 \pm 0.56b	63.4 \pm 1.02b
28	<i>C. tripunctatus lateralis</i>	21	6.9 \pm 0.42a	6.5 \pm 0.25a	8.1 \pm 0.43a	32.7 \pm 1.82a	54.2 \pm 2.51a
	<i>C. brevis</i>	14	8.6 \pm 0.43b	9.4 \pm 0.56b	10.3 \pm 0.37b	29.3 \pm 1.75a	57.6 \pm 1.40a
	<i>C. chinensis</i>	14	6.1 \pm 0.38a	7.2 \pm 0.33a	12.3 \pm 0.42c	32.9 \pm 3.45a	58.4 \pm 3.32a
30	<i>C. tripunctatus lateralis</i>	39	8.1 \pm 0.47a	7.5 \pm 0.35a	8.5 \pm 0.33a	26.3 \pm 0.73a	50.3 \pm 1.48a
	<i>C. brevis</i>	16	9.3 \pm 0.41a	8.5 \pm 0.71a	9.8 \pm 0.58a	28.3 \pm 1.90a	55.9 \pm 2.53a
	<i>C. chinensis</i>	20	6.9 \pm 0.50a	6.9 \pm 0.39a	12.7 \pm 0.37b	26.2 \pm 0.74a	52.7 \pm 1.21a

2 Means within a temperature and within a stage followed by different letters are significantly different among species (Tukey-Kramer test: $P < 0.05$).

3

Table 3 Regression line, developmental zero, and effective accumulated temperature in each of the three *Cybister* species

Species	Stage	R ²	b*	a*	Developmental Zero (°C)	Effective accumulated temperature (degree-days)
<i>C. tripunctatus</i>	Egg	0.896	0.0070	-0.0820	11.7	142.9
<i>lateralis</i>	1st instar	0.984	0.0170	-0.3281	19.3	58.8
	2nd instar	0.975	0.0185	-0.3625	19.6	54.1
	3rd instar	0.971	0.0062	-0.0511	8.2	161.3
	Pupa	0.935	0.0023	-0.0343	14.9	434.8
	1st instar-adult	0.996	0.0016	-0.0269	16.8	625.0
<i>C. brevis</i>	Egg	0.966	0.0057	-0.0595	10.4	175.4
	1st instar	0.921	0.0085	-0.1148	13.5	117.6
	2nd instar	0.762	0.0065	-0.0581	10.5	153.8
	3rd instar	0.945	0.0041	-0.0155	3.8	243.9
	Pupa	0.923	0.0016	-0.0101	6.3	625.0
	1st instar-adult	0.949	0.0011	-0.0122	11.1	909.1
<i>C. chinensis</i>	Egg	0.992	0.0076	-0.1052	13.8	131.6
	1st instar	0.990	0.0101	-0.1209	12.0	99.0
	2nd instar	0.976	0.0081	-0.0851	10.5	123.5
	3rd instar	0.993	0.0033	-0.0114	3.5	303.0
	Pupa	0.901	0.0017	-0.0142	8.4	588.2
	1st instar-adult	0.961	0.0009	-0.0078	8.7	1111.1

* $1/D = a + bT$, where D is developmental period, T is temperature, a is an intercept of the regression line, b is a coefficient. Regression equations calculated based on the developmental periods obtained from 20 to 28 °C in *C. brevis* and *C. chinensis* and 23-28°C in *C. tripunctatus lateralis* (All regression analysis is significant ($p < 0.05$)).

Figure 1

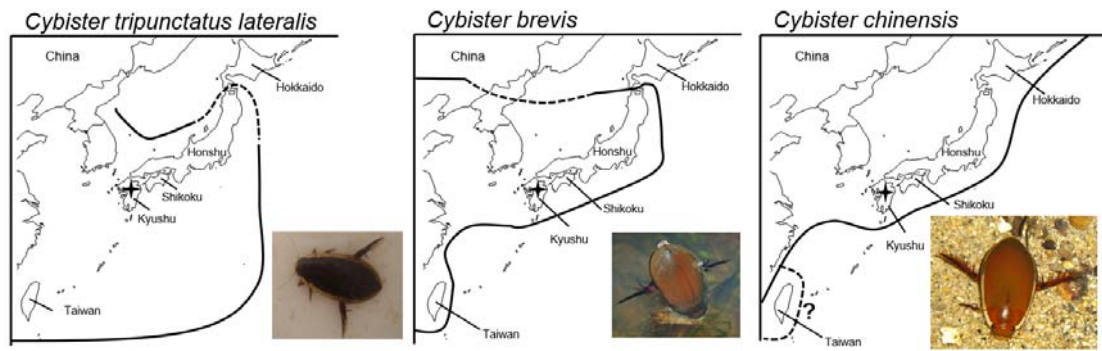


Figure 2

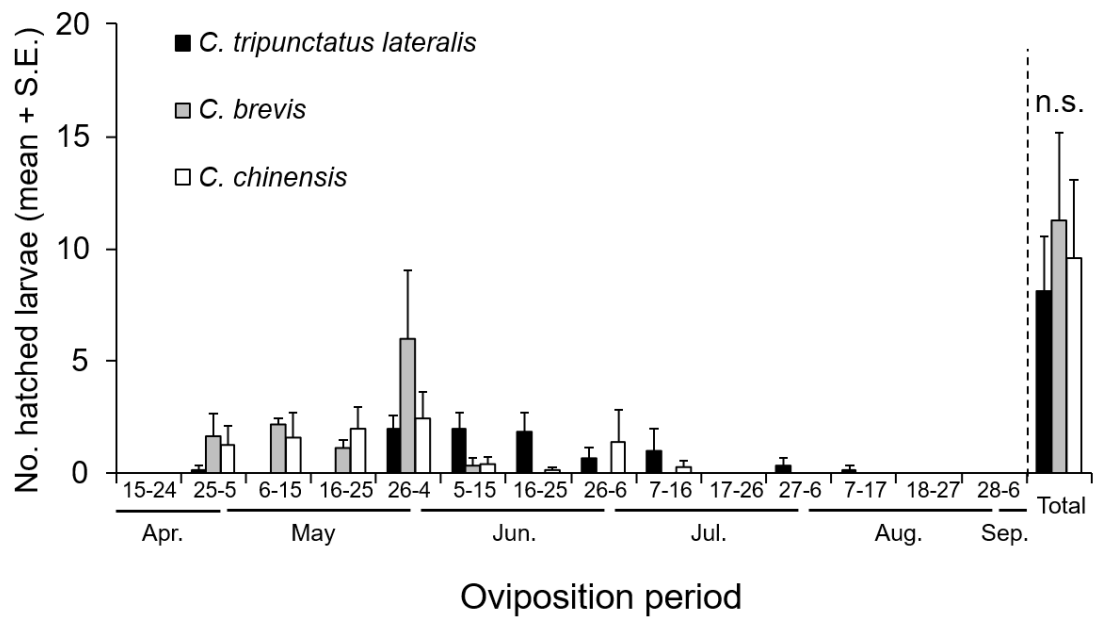


Figure 3

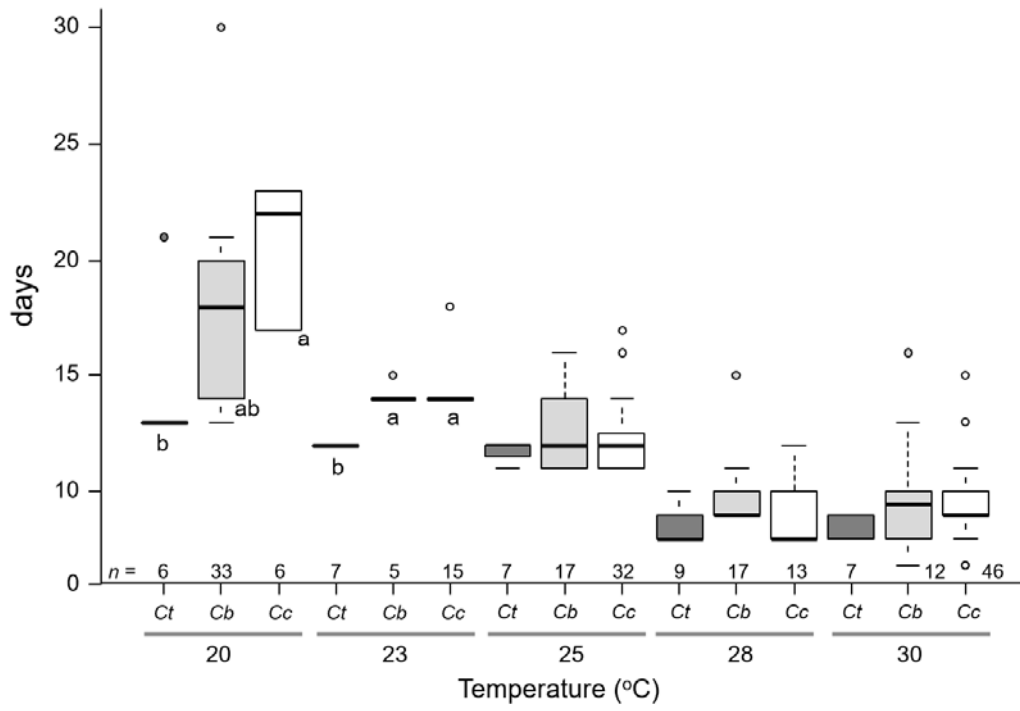


Figure 4

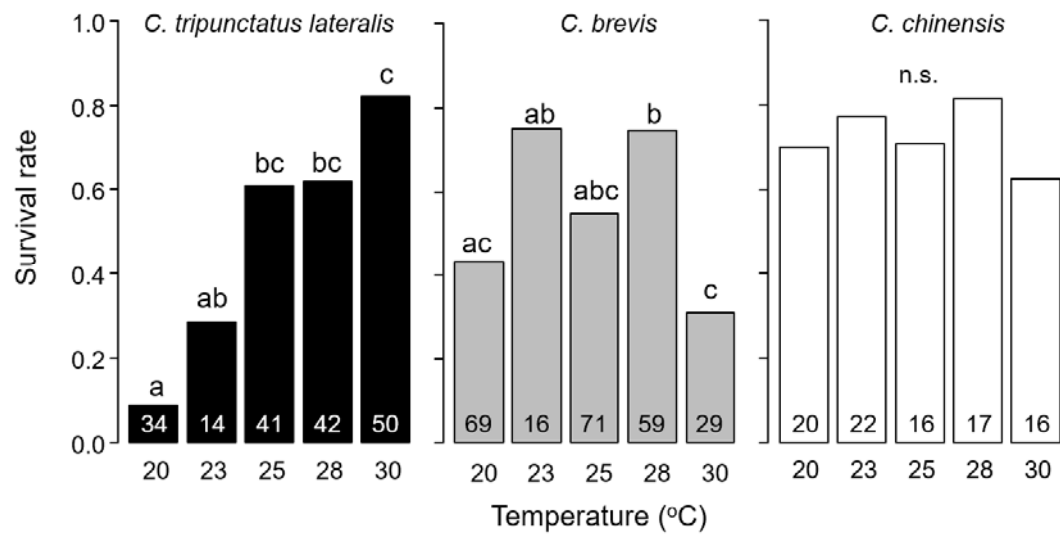
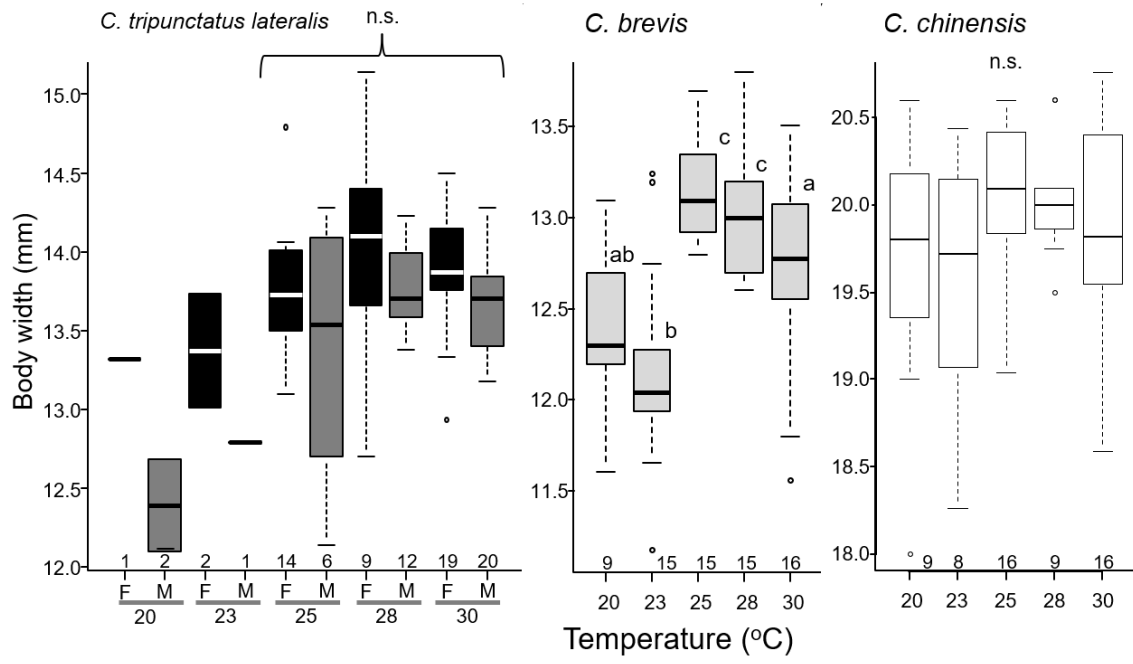


Figure 5



Supporting Information

Table S1 Size of plastic cup used in this rearing experiment

Species	First instar	Second instar	Third instar
<i>Cybister tripunctatus lateralis</i>	A	A	B
<i>C. brevis</i>	A	A	B
<i>C. chinensis</i>	A	B	B

A: 101 mm diameter, 45 mm high

B: 129 mm diameter, 60 mm high

Table S2 Size (mm) of frozen cricket fed to diving beetle larva

Species	First instar	Second instar	Third instar
<i>Cybister tripunctatus lateralis</i>	5-8	8-10	10-12
<i>C. brevis</i>	5-8	8-10	10-12
<i>C. chinensis</i>	8-10	10-12	15-20

Table S3 Comparison of coefficient (a) and intercept (b) of the regression line and developmental zero between different temperature regions

Species	Temperature (°C)	Stage	R ²	b*	a*	Developmental zero	Temperature (°C)	R ²	b*	a*	Developmental zero
<i>Cybister tripunctatus</i> <i>lateralis</i>	23, 25, 28, 30	1st	0.709	0.0100	-0.1555	15.6	23, 25, 28	0.984	0.0170	-0.3281	19.3
		2nd	0.743	0.0113	-0.1862	16.5		0.975	0.0185	-0.3625	19.6
		3rd	0.831	0.0042	-0.0026	0.6		0.971	0.0062	-0.0511	8.2
		pupa	0.961	0.0027	-0.0449	16.6		0.935	0.0023	-0.0343	14.9
		all	0.982	0.0014	-0.0219	15.6		0.996	0.0016	-0.0269	16.8
<i>C. brevis</i>	20, 23, 25, 28, 30	1st	0.767	0.0060	-0.0579	9.7	20, 23, 25, 28	0.921	0.0085	-0.1148	13.5
		2nd	0.820	0.0058	-0.0541	9.3		0.762	0.0065	-0.0681	10.5
		3rd	0.955	0.0037	-0.0078	2.1		0.945	0.0041	-0.0155	3.8
		pupa	0.951	0.0019	-0.0189	9.9		0.923	0.0016	-0.0101	6.3
		all	0.922	0.0009	-0.0083	9.2		0.949	0.0011	-0.0122	11.1
<i>C. chinensis</i>	20, 23, 25, 28, 30	1st	0.838	0.0073	-0.0565	7.7	20, 23, 25, 28	0.990	0.0101	-0.1209	12.0
		2nd	0.965	0.0071	-0.0642	9.0		0.976	0.0081	-0.0851	10.5
		3rd	0.897	0.0026	0.0061	-2.3		0.993	0.0033	-0.0114	3.5
		pupa	0.941	0.0019	-0.0192	10.1		0.901	0.0017	-0.0142	8.4
		all	0.975	0.0009	-0.0070	7.8		0.962	0.0009	-0.0078	8.7

*1/D = a + bT, where D is developmental period, T is temperature, a is an intercept of the regression line, b is a coefficient.

Bold typeface indicates higher R² values and their developmental zero.

Table S4 Oviposition pattern in the three *Cybister* species

Species	Pair	15-24 Apr	25 Apr-5 May	6-15 May	16-25 May	26 May-4 Jun	5-15 Jun	16-25 Jun	26 Jun-6 Jul	7-16 Jul	17-26 Jul	27 Jul-6 Aug	7-17 Aug	18-27 Aug	28 Aug-6 Sep	Total
<i>C. brevis</i>	A	0	0	3	2	14	2	0	0	0	0	0	0	0	0	21
	B	0	3	2	1	17	0	0	0	0	0	0	0	0	0	23
	C	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
	D	0	6	3	2	3	0	0	0	0	0	0	0	0	0	14
	E	0	0	2	1	0	0	0	0	0	0	0	0	0	0	3
	F	0	1	2	1	2	0	0	0	0	0	0	0	0	0	6
<i>C. chinensis</i>	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	0	0	5	1	0	10	2	0	0	0	0	0	18
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	5	8	5	3	0	0	0	0	0	0	0	0	0	21
	F	0	4	3	3	1	2	0	0	0	0	0	0	0	0	13
	G	0	0	0	6	8	0	1	0	0	0	0	0	0	0	15
<i>C. tripunctatus</i> <i>lateralis</i>	A	0	0	0	0	2	2	2	3	0	0	0	0	0	0	9
	B	0	0	0	0	2	1	4	1	6	0	0	0	0	0	14
	C	0	0	0	0	4	5	5	0	0	0	0	1	0	0	15
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	E	0	1	0	0	1	1	0	0	0	0	0	0	0	0	3
	F	0	0	0	0	3	3	0	0	0	0	2	0	0	0	8