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ORIGINAL ARTICLE

Comparison of behavior and foraging ability between two congeneric species of large-bodied diving beetle (Coleoptera: Dytiscidae) larvae, a non-expanding species and a distribution-expanding species

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Abstract. Global warming has altered the distribution of several insect species, including those inhabiting aquatic environments and this range expansion may modify existing interspecific interactions by facilitating new encounters between species that were not originally sympatric. Global warming is highly likely to expand the distribution of *Cybister tripunctatus lateralis* (Coleoptera: Dytiscidae) in Japan, leading to interspecific competition for food resources among congeneric species. In particular, *Cybister* larvae share similar feeding habits, suggesting that interspecific competition is likely to occur, although this has not yet been verified. Therefore, this study aimed to compare the microhabitat use and foraging abilities of the larvae of two species – *C. tripunctatus lateralis* with an expanded distribution area and *Cybister brevis*, a non-expanding species of similar body size. We compared (1) behavior quantified by visual scan census, (2) time taken to reach food, and the number of prey animals consumed by each species in the laboratory experiments. The results indicated that the behavioral patterns of the larvae tended to involve mainly perch and rest on the plants. There were no clear differences between the two species, although there were variations among the instars. There was no difference in the number of prey animals consumed by the two species; however, it was evident that *C. tripunctatus lateralis* reached the food earlier than *C. brevis*. Therefore, it is suggested that the two species utilize a common underwater microhabitat, and that *C. tripunctatus lateralis* has a competitive advantage over *C. brevis* in terms of food resources. However, further experimental verification and continued expansion of *C. tripunctatus lateralis* distribution trends should be carefully monitored, as reproductive habitats in the field differ and competitive advantages may depend on water temperature.

INTRODUCTION

Global warming has had significant ecological impacts on insects (Menéndez, 2007; Robinet & Roques, 2010), resulting in changes in the distribution and poleward expansion of several taxa (Hickling et al., 2006). Some studies have specifically focused on aquatic insects (Li et al., 2014; Navara et al., 2020), and distribution expansion has been reported for dragonflies (Hickling et al., 2005; Hassall & Thompson, 2008; Ott, 2010), caddisflies (Sáinz-Bariáin et al., 2016; Navara et al., 2020), and stone flies (Bojková et al., 2012). Diving beetle species (Coleoptera: Dytiscidae), such as *Cybister lateralimarginalis* (De Geer, 1774) (Thomas, 2009; Petrov & Fedorova, 2013), *Rhantus suturalis* (Macleay, 1825) (Bilton, 2023), *Eretes sticticus* (Linnaeus, 1767) (Hajek et al., 2014; Nitzu, 2022), *Hydrovatus cuspidatus* (Kunze, 1818) (Scheers, 2014, 2017), and *Graptodytes bilineatus* (Sturm, 1835) (Scheers, 2014), are expanding their distribution. The expansion of their distribution may lead to new interspecific interactions because as they encounter species that are not originally sympatric (Menéndez, 2007; Robinet & Roques, 2010; Shah et al., 2020; Nagano et al., 2023). Therefore, there is a need to accumulate knowledge on the persistence of species and the potential loss of biodiversity due to changes in interspecific interactions caused by global warming (Menéndez, 2007).

Diving beetles which are the top invertebrate predators in most fishless freshwater habitats, feed on a variety of aquatic organisms, such as zooplankton, aquatic insects, and amphibians (Culler et al., 2023). In Japan, 45% of the 27/60 species of Dytiscidae inhabiting paddy field water

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systems are listed on the Red List (Ministry of the Environment of Japan, 2020; Nakajima et al., 2020). These include seven large-bodied species of the genus *Cybister* (adult body length > 20 mm), six of which are also listed on the Red List (Ministry of the Environment of Japan, 2020). The factors behind this endangerment are considered to include dry paddy fields, agricultural ditch renovation, pesticide pollution, and abandonment of cultivation (Nishihara et al., 2006; Ichikawa, 2008). *Cybister brevis* Aubé, 1838, distributed in Honshu, Shikoku, and Kyushu, is listed as "near threatened" on the Red List of Japan due to its declining population. However, the distribution of *Cybister tripunctatus lateralis* (Fabricius, 1798), a tropical and subtropical species found in the Honshu, Shikoku, Kyushu, and Ryukyu Islands, is expanding northward (Shimono, 2015; Nakajima et al., 2020), its status on the Red List of Japan was revised from "critically endangered" to "vulnerable" in 2012 (Ministry of the Environment of Japan, 2015).

Cybister tripunctatus lateralis is expanding its distribution for several reasons. The lower developmental temperature limit for first instar larvae to adults was the highest for *C. tripunctatus lateralis* (16.8°C) compared to *C. brevis* (11.1°C) and *Cybister chinensis* Motschulsky, 1854 (8.7°C), while the survival of *C. brevis* peaked at 23–28°C, *C. tripunctatus lateralis* at 30°C and the survival of *C. chinensis* was independent of the temperature under 30°C (Ohba et al., 2020). Furthermore, adults *C. tripunctatus lateralis* have been observed to die during overwintering when the water temperature drops below freezing. However, in recent years, the average minimum temperature during the coldest months in regions where *C. tripunctatus lateralis* occurs has remained above freezing (Ohba et al., 2023). Thus, the temperature required for the growth and survival of *C. tripunctatus lateralis* is higher than that required for congeneric species, suggesting that the distribution of this species can expand under global warming. This expansion may be further enhanced by a higher flying frequency/ability of *C. tripunctatus lateralis* compared to congeneric species, which is likely a factor in its recent distributional expansion (Ohba et al., 2025).

This expanding distribution may lead to interspecific competition with congeneric species (Watanabe, 2021; Ohba et al., 2022). In adults, *C. tripunctatus lateralis* swims more actively, finds food earlier, and feeds more than congeneric species, suggesting the possibility of interspecific competition for food (Ohba et al., 2022). However, in Dytiscidae adults, evidence of competition for food resources is lacking between two sympatric *Laccophilus* species (Pitcher & Yee, 2014) and two *Hygrotus* species (Juliano & Lawton, 1990) in laboratory experiments. In addition, five floodplain-dwelling *Neoporus* species showed no differences in phenology or habitat (McDaniel et al., 2017). In contrast, competition or density-dependent cannibalism has been observed among larvae (Juliano & Lawton, 1990), and several studies have suggested interspecific coexistence through niche partitioning. Laboratory experiments have suggested that larval coexistence is facilitated by niche differentiation, such as variations in hunting modes (Yee, 2010) and the use of different prey and microhabitats depending on specific hunting modes (Yee et al., 2013). Field observations have also suggested that four species of Dytiscidae larvae coexist in paddy fields because of differences in phenology, prey size, prey preference, and spatial positioning in the water column (underwater position) (Watanabe et al., 2024). This contrast may be explained by the fact that adults are scavengers with the ability to fly, whereas larvae are exclusive predators with limited dispersal ability.

Although the reproductive habitat use of *C. brevis* and *C. tripunctatus lateralis* differs in paddy field water systems (*C. brevis* larvae are more abundant in agricultural ditches, while *C. tripunctatus lateralis* are more common in paddy fields), their phenology and underwater positions overlap (Fukuoka et al., 2024). Furthermore, the larvae of the two species are approximately the same size (50 mm in the third instar; Mitamura et al., 2017; Fig. 1) and prey on aquatic insects, such as dragonfly nymphs and water boatmen (Ohba, 2009; Ohba & Inatani, 2012; Ohba & Ogushi, 2020; Fukuoka et al., 2021). Thus, the niche overlap and expanding distribution of *C. tripunctatus lateralis* may result in interspecific competition for food resources with *C. brevis*.

This study compared the differences in microhabitat use and foraging ability between *C. tripunctatus lateralis* larvae, a species that is expanding its distribution, and *C. brevis* larvae, a non-expanding species. We predicted (1) based on field observations by Fukuoka et al. (2024), that

Fig. 1. Third instar larvae of *C. brevis* (a), a non-expanding species, and *C. tripunctatus lateralis* (b), a distribution-expanding species.

there would be no difference in the behavior of the two species, and (2) based on competition experiments with adults conducted by Ohba et al. (2022), that *C. tripunctatus lateralis* would outcompete *C. brevis* in terms of the number of prey consumed, and the time taken to reach the food, and that a similar pattern would also be observed in larvae.

MATERIAL AND METHODS

The larvae used in this experiment were obtained by breeding them from three pairs of *C. brevis* adults collected in April 2020 in a paddy field in Takashima City, Shiga Prefecture, and from three pairs of *C. tripunctatus lateralis* adults collected in April 2020 in a natural wetland in Isahaya City, Nagasaki Prefecture. Each collection site contained only one *Cybister* species and was not sympatric. As in Fukuoka et al. (2023), larvae were reared individually in tea strainers (6.5 cm diameter, 6.5 cm high, 1 mm mesh) placed in a plastic container (53 cm long, 34.5 cm wide, 9 cm high (internal dimensions)) maintained at 3–4 cm depth and 25–28°C water temperature. Since *Cybister* larvae can grow solely on a diet of crickets *Gryllus bimaculatus* De Geer, 1773 (Orthoptera: Gryllidae) (Ohba et al., 2020; Watanabe et al., 2021), frozen crickets of each size were fed two to three times a day depending on the larval stage (first and second instars: 8–10 mm, third instar: 20–25 mm (body length); Tsukiyono Farm, Gunma, Japan).

This experiment was designed to compare the behavior and foraging abilities of the two species. However, direct competition experiments, including interspecific predation, were not conducted. Therefore, each species was tested separately for two reasons: (1) *Cybister* larvae indiscriminately prey on each other indiscriminately and (2) the reproductive periods of the two species did not coincide, making it difficult to obtain larvae at the same growth stage. Three types of experiments were conducted between 10:00 and 15:00 from May to July 2020. The water temperature in the experimental vessels was maintained at 25–28°C. Larvae were used in the experiments 2–3 days after hatching or molting and were fasted for 24 h prior to the experiments to standardize hunger level. Each larva was used only once in the experiment for each instar. All the experiments were performed by a single observer (T. Fukuoka). All the statistical analyses for the following experiments were performed using the R software version 4.4.2 (R Core Team, 2024).

Quantifying behavior

The position, location, and activity of the larvae were recorded for 30 min to compare the microhabitat use of the two species. A plastic container (15 × 22 × 16 cm, length × width × height) was placed with one tree branch (length: 20 cm) standing diagonally as a scaffold for the larvae and one *Egeria densa* Planch. plant (length: 20 cm) (Fig. S1). The larvae were placed in containers and allowed to acclimate for 10 min. The following variables were recorded every minute for 30 min by visual observation according to the method described in Yee (2010): the position of the larvae was recorded as "surface" when they were in contact with the water surface and "underwater" when they were in the middle or bottom of the water column. The location of the larvae was recorded as "plant" when they were clinging to *E. densa* or a tree branch, and "space" when they were not clinging to any object. The activity was recorded as "move" when the larvae were walking or swimming, and "rest" when they were stationary. The water depth was set at approximately two times the total length of the larvae (first instar: 4 cm; second instar: 6 cm; and third instar: 10 cm) to facilitate behavioral observations in water. The sample sizes at each instar for the two species were as follows – *C. brevis* first instar: *n* = 23; second instar: *n* = 26; third instar: *n* = 31; *C. tripunctatus lateralis* first instar: *n* = 23, second instar: *n* = 20; third instar: *n* = 36.

We analyzed the data using a generalized linear mixed model (GLM) with a negative binomial distribution, with the number of observations on one side (surface, plant, and move) as the response variable and species, instar, and their interactions as explanatory variables. The "MASS" package version 7.3.61 (Venables & Ripley, 2002) was used for GLM with a negative binomial distribution.

In addition, we also examined individual behavior using principal component analysis (PCA), as shown in Yee (2010). Six variables – position: surface, underwater; location: plant, space; and activity: move, rest – were transformed into ratios from 30 observations and then arcsine-transformed to homogenize the variance. A PCA was performed using the obtained values. The PCA results revealed that PC1 and PC2 accounted for 76.6 and 17.4% of the total variation, respectively, and together explained 94.0% of the total variation (Table S1). The PC1 score did not meet the assumption of normality, we performed a Scheirer-Ray-Hare test (Scheirer et al., 1976), a nonparametric two-way analysis of variance (ANOVA) with the PC1 score as the response variable, and species and instar, and their interactions as explanatory variables. The "rcompanion" package version 2.5.0 (Mangiafico, 2025) was used for Scheirer-Ray-Hare test.

Foraging ability

Time taken to reach food

To compare the food-searching abilities of the two species, observation was carried out during an hour. The larvae were placed in plastic containers (30 × 8 × 7 cm) and allowed to acclimate for 10 min. One frozen cricket (body length: 8–10 mm) was then placed on the water surface, maintaining a distance of 5 cm from the head of the larvae to minimize disturbance (Fig. S2). The larvae were observed for 1 h and the time (in seconds) taken to capture *G. bimaculatus* was measured. The water depth was maintained at 1, 2, and 3 cm for the first, second, and third instars, respectively, to ensure that the larvae could move along the bottom. The sample sizes for each instar in both species were as follows – *C. brevis* first instar: *n* = 18; second instar: *n* = 19; third instar: *n* = 21; *C. tripunctatus lateralis* first instar: *n* = 15, second instar: *n* = 17; third instar: *n* = 33.

Larvae that reached the food within 1 h (food discovery rate) accounted for 95.3% of all individuals. using a GLM with a binomial distribution in which the food discovery rate as the response variable and species, instar, and their interactions as explanatory variables. No significant effects of species, instars, or their interactions were observed (Table S2). The data from individuals who could not reach the food within 1 h were, therefore, excluded from subsequent analyses. We analyzed the data using a GLM assuming a gamma distribution with time to reach food (seconds) as the response variable and species, instar, and their interaction as explanatory variables.

Number of prey animals consumed

The number of prey animals consumed by the larvae over 24 h was recorded to compare the food requirements of the two species. The larvae were acclimated for 10 min in a plastic container (15 cm × 22 cm × 16 cm) containing one *E. densa* plant (length: 20 cm). Prey animals consistent with their natural diet in the field were introduced, based on the results of Ohba (2009), Ohba & Ogushi (2020), and Fukuoka et al. (2021) (Fig. S3-a). The first instar larvae were provided with 30 *Asellus hilgendorfi* Bovallius, 1886 (Isopoda: Asellidae) (body length: 5–10 mm), the second instar larvae with 10 damselfly nymphs (Odonata: Zygoptera)

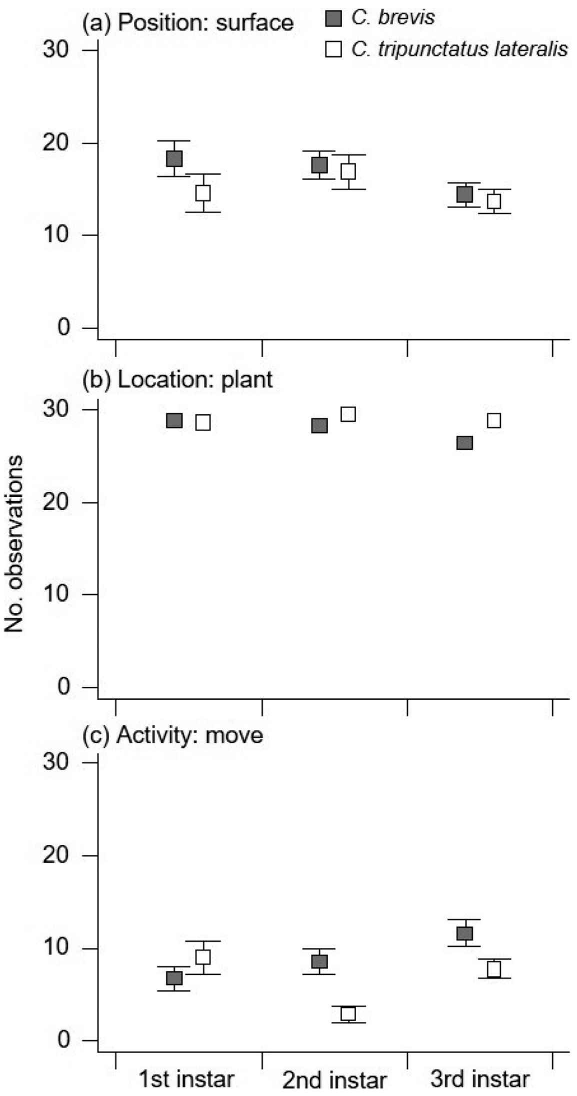

Fig. 2. Number of observations (mean ± SE) of position (surface) (a), location (plant) (b), and activity (move) (c) for the two species, out of a total of 30 observations. The SE bars in panel (b) are not displayed due to the very small values.

(body length: 15–20 mm), and the third instar larvae with 10 dragonfly nymphs (Odonata: Libellulidae) (body length: 20–30 mm). The number of prey consumed by the larvae was recorded after 24 h. Prey eaten by the larvae lose body color (red arrows: eaten, black arrows: alive, Fig. S3-b), which allowed determination of whether they were consumed. There were no instances of predation among the prey or natural deaths during the experiment. The water depth was maintained at 1, 2, and 3 cm for the first, second, and third instar larvae, respectively. The sample sizes at each instar for the two species were as follows – *C. brevis* first instar: *n* = 12; second instar: *n* = 13; third instar: *n* = 20; *C. tripunctatus lateralis* first instar: *n* = 14, second instar: *n* = 20; third instar: *n* = 21.

We compared the number of prey consumed between the species using GLMs with a Poisson distribution. The analysis was conducted separately for each instar, as the types of prey provided differed among instars.

RESULTS

Quantifying behavior

The larvae of both species mainly perched on the plant (Fig. 2). The GLM results for each behavioral variable showed a significant effect of the interaction between species and instar in activity, whereas the species and instar, and their interaction was not significant in position and location (Table 1). For the first instar, *C. tripunctatus lateralis* moved more actively than *C. brevis*, whereas for the second and third instars, *C. brevis* moved more actively than *C. tripunctatus lateralis* (Fig. 2-c).

The PCA results showed that the factor pattern for PC1 had strong positive coefficients (> 0.35) for water surface, plants, and rest, and strong negative coefficients (> –0.35) for underwater, space, and movement (Table S1). For *C. brevis*, the PC1 scores decreased as instar numbers increased (Fig. S4). For *C. tripunctatus lateralis*, the PC1 scores increased only at the second instar stage, with intermediate scores at the first and third instars (Fig. S4). The Scheirer-Ray-Hare test results showed a significant effect of instar, while species and their interaction were not significant (species: $H_{1,153}$ = 1.674, P = 0.196; instar: $H_{2,153}$ = 6.467, P = 0.039; species × instar: $H_{2,153}$ = 4.718, P = 0.095). Therefore, although there was variation among the instars, no clear difference in the behavioral patterns was found between the two species.

Time taken to reach food

The GLM results showed a significant effect of species, while instar and their interaction were not significant (Table 2). Therefore, *C. tripunctatus lateralis* took significantly less time to reach the food than *C. brevis* (Fig. 3).

Table 1. Results of fitting a generalized linear model (GLM) to each behavioral variable at each instar.

	Estimate	SE	z	P-value
Location (response variable: surface)				
(Intercept)	−0.49	0.11	–4.38	<0.0001
C. tripunctatus lateralis [a]	−0.23	0.16	–1.42	0.156
2nd instar [b]	–0.04	0.15	–0.25	0.803
3rd instar [c]	–0.24	0.15	–1.59	0.111
C. tripunctatus lateralis: 2nd instar	0.18	0.23	0.81	0.418
C. tripunctatus lateralis: 3rd instar	0.17	0.21	0.83	0.408
Position (response variable: plant)				
(Intercept)	–0.04	0.04	–0.99	0.322
C. tripunctatus lateralis [a]	–0.005	0.05	–0.08	0.934
2nd instar [b]	–0.02	0.05	–0.29	0.771
3rd instar [c]	–0.09	0.05	–1.63	0.103
C. tripunctatus lateralis: 2nd instar	0.05	0.08	–0.58	0.562
C. tripunctatus lateralis: 3rd instar	0.09	0.07	1.27	0.206
Activity (response variable: move)				
(Intercept)	–1.48	0.23	6.56	<0.0001
C. tripunctatus lateralis [a]	0.28	0.32	0.89	0.375
2nd instar [b]	0.24	0.31	0.77	0.441
3rd instar [c]	0.54	0.29	1.82	0.068
***C. tripunctatus lateralis*: 2nd instar**	**–1.36**	**0.46**	**–2.95**	**0.003**
C. tripunctatus lateralis: 3rd instar	–0.68	0.41	–1.66	0.097

[a] The coefficient indicates the relative effect of *C. tripunctatus lateralis* compared with *C. brevis*. [b] The coefficient indicates the relative effect of second instar compared with first instar. [c] The coefficient indicates the relative effect of third instar compared with first instar.

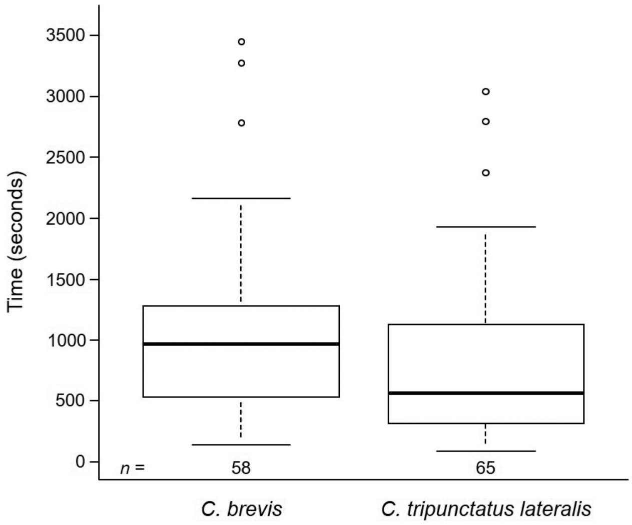

Fig. 3. Time (seconds) taken by the two species to reach food. The GLM results showed that the effect of instar was not significant (Table 2); therefore, the data were pooled. The thick line, the upper and lower boxes, and the whiskers indicate the median, the first and third quartile, and 1.5× the interquartile range, respectively; the dots are outliers. Figures just above the horizontal axis indicate the sample sizes.

For *C. brevis*, the time taken to reach the food was 1124 ± 765 s (mean ± SD) in the first instar, 1034 ± 705 s in the second instar, and 949 ± 666 s in the third instar. For *C. tripunctatus lateralis*, it was 636 ± 475 s in the first instar, 686 ± 448 s in the second instar, and 907 ± 781 s in the third instar.

Number of prey animals consumed

The GLM showed that the number of prey animals consumed each instar was not significantly different between the species (Table 3; Fig. 4). The number of prey animals consumed by *C. brevis* was 16.7 ± 3.2 (mean ± SD) *Asellus hilgendorfi* in the first instar, 4.5 ± 1.1 damselfly nymphs (Odonata: Zygoptera) in the second instar and 4.6 ± 1.6 dragonfly nymphs (Odonata: Libellulidae) in the third instar. The number of prey animals consumed by *C. tripunctatus lateralis* was 18.0 ± 5.0 *Asellus hilgendorfi* in the first instar, 5.1 ± 1.1 damselfly nymphs (Odonata: Zygoptera) in the second instar, and 5.1 ± 1.3 dragonfly nymphs (Odonata: Libellulidae) in the third instar.

Table 2. Results from fitting a generalized linear model (GLM) to the time taken to reach food at each instar.

	Estimate	SE	z	P-value
(Intercept)	7.02	0.17	40.18	<0.0001
C. tripunctatus lateralis [a]	**−0.57**	**0.26**	**−2.20**	**0.030**
2nd instar [b]	−0.10	0.24	−0.42	0.677
3rd instar [c]	−0.15	0.24	−0.61	0.545
C. tripunctatus lateralis: 2nd instar	0.18	0.36	0.50	0.620
C. tripunctatus lateralis: 3rd instar	0.50	0.33	1.50	0.135

[a] The coefficient indicates the relative effect of *C. tripunctatus* lateralis compared with *C. brevis*. [b] The coefficient indicates the relative effect of second instar compared with first instar. [c] The coefficient indicates the relative effect of third instar compared with first instar.

Table 3. Results of the generalized linear model (GLM) fitted to the number of prey animals consumed at each instar.

	Estimate	SE	z	P-value
1st instar				
(Intercept)	2.81	0.071	39.79	<0.0001
C. tripunctatus lateralis [a]	0.08	0.095	0.81	0.416
2nd instar				
(Intercept)	1.50	0.131	11.39	<0.0001
C. tripunctatus lateralis [a]	0.13	0.165	0.81	0.416
3rd instar				
(Intercept)	1.52	0.105	14.45	<0.0001
C. tripunctatus lateralis [a]	0.11	0.143	0.79	0.427

[a] The coefficient indicates the relative effect of *C. tripunctatus lateralis* compared with *C. brevis*.

DISCUSSION

Although global warming has led to the suggested distributional expansion of some Dytiscidae species (Thomas, 2009; Petrov & Fedorova, 2013; Hajek et al., 2014; Scheers, 2014, 2017; Nitzu, 2022; Bilton, 2023), little is known about its impact on native species. Previous studies have suggested that *C. tripunctatus lateralis*, which is expanding its distribution, may compete with congeneric species (*C. brevis* and *C. chinensis*) for food resources (Ohba et al., 2022). However, this experiment was conducted on adults and not on larvae. This study compared the microhabitat use and foraging abilities of *C. tripunctatus lateralis* and *C. brevis*. The behavioral patterns of the larvae were mainly perching and resting on the plants. Although there was variation among the instars, which became more active as the instars progressed, there was no clear difference between the two species. Regarding foraging ability, there was no difference in the number of prey animals consumed by the two species, but *C. tripunctatus lateralis* reached food earlier than *C. brevis*. Our predictions were generally supported, except for the number of prey consumed.

There was no difference in the underwater positions of the two species in paddy fields, and most individuals were observed at the bottom of the water or perched on plants (Fukuoka et al., 2024). Our results support the use of microhabitats in this field. In addition, the larvae of the two species are present from June to August (Fukuoka et al., 2024) and prey on aquatic insectivores, such as dragonfly nymphs and water boatmen (Ohba, 2009; Ohba & Inatani, 2012; Ohba & Ohgushi, 2020; Fukuoka et al., 2021). Due to this overlap in appearance season and food resources, the ability of *C. tripunctatus lateralis* to access food earlier than *C. brevis* may provide a competitive advantage when food is limited.

Dytiscidae larvae exhibit different hunting modes, depending on the species. *Dytiscus* larvae remain still and ambush their prey, *Graphoderus* larvae actively swim, and *Rhantus* larvae exhibit a combination of these behaviors (Yee, 2010). *Graphoderus* and *Rhantus*, which share the same habitat, are believed to coexist due to the differences in prey species and microhabitats resulting from these hunting modes (Yee et al., 2013). Furthermore, *Hydaticus bowringii* Clark, 1864 and *C. brevis* larvae in paddy

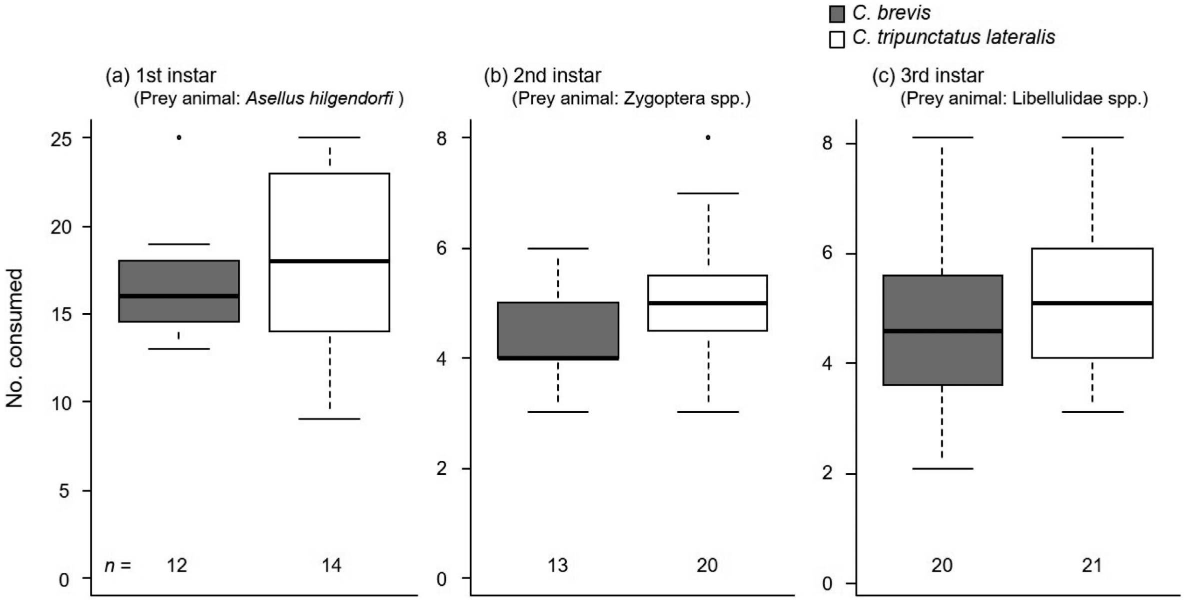

Fig. 4. Number of prey animals (*Asellus hilgendorfi* (a), Zygoptera spp. (b) and Libellulidae spp. (c)) consumed in 24 h at each instar for the two species. The thick line, the upper and lower boxes, and the whiskers indicate the median, the first and third quartile, and 1.5× the interquartile range, respectively; the dots are outliers. Figures just above the horizontal axis indicate the sample sizes.

fields do not differ in their microhabitat use in water but are thought to coexist due to differences in feeding habits and phenology (Watanabe et al., 2024). These coexistence mechanisms have not been identified for *C. brevis* and *C. tripunctatus lateralis*. In contrast, the reproductive habitat use of the two species differed, with *C. brevis* larvae appearing more abundantly in agricultural ditches than *C. tripunctatus lateralis* larvae in paddy fields (Fukuoka et al., 2024). In general, *C. brevis* also uses paddy fields as its reproductive habitat (e.g., Saijo, 2001; Ohba, 2009; Fukuoka et al., 2021; Watanabe et al., 2024), suggesting that interspecific competition in larvae may be restricted to paddy fields.

Furthermore, changes in water temperature are important factors in understanding coexistence mechanisms, as they influence the intensity of interactions between predatory aquatic insects. Specifically, prey consumption (Frances & McCauley, 2018; Mira-Mendes et al., 2019; Twardochleb et al., 2020; Nagano et al., 2023), aggression (Frances & McCauley, 2018; Twardochleb et al., 2020; Nagano et al., 2023), and activity (Twardochleb et al., 2020), all of which are associated with interspecific competition, increase with rising water temperature. In laboratory experiments, it has revealed that foraging intake of *Trithemis aurora* (Burmeister, 1839), which is expanding owing to global warming, increases with rising temperatures and reduces that of the native *Orthetrum albistylum speciosum* (Uhler, 1858) (Nagano et al., 2023). *Cybister brevis* is distributed at higher latitudes than *C. tripunctatus lateralis* (Nakajima et al., 2020) and has a lower developmental temperature limit (Ohba et al., 2020). Therefore, competitive advantage depends on temperature, and different environmental factors (e.g., illumination and elevation) in the habitat may facilitate coexistence. In the present study, the water temperature was maintained within the range of 25–28°C. However, further experiments at different temperatures are needed to clarify this relationship. Additionally, since the present study was conducted in a simple plastic container in a laboratory setting, caution must be exercised when applying the results to a field environment. Thus, it is important to evaluate the results of mesocosm experiments in which the presence of predators and prey is controlled.

Progressive global warming is expected to continue to expanding the distribution of *C. tripunctatus lateralis* (Ohba et al., 2023) and may intensify interspecific competition with *C. brevis*. Ongoing distribution surveys should be conducted, and trends carefully monitored.

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REFERENCES

Bilton D.T. 2023: Chapter 11: Dispersal in Dytiscidae. In Yee D.A. (ed.): *Ecology, Systematics, and the Natural History of Predaceous Diving Beetles (Coleoptera: Dytiscidae), 2nd ed.* Springer, Cham, pp. 505–528.

Bojková J., Komprdová K., Soldán T. & Zahrádková S. 2012: Species loss of stoneflies (Plecoptera) in the Czech Republic during the 20th century. — *Freshw. Biol.* **57**: 2550–2567.

Culler L.E., Ohba S. & Crumrine P. 2023: Chapter 8: Predator-prey ecology of Dytiscids. In Yee D.A. (ed.): *Ecology, Systematics, and the Natural History of Predaceous Diving Beetles (Coleoptera: Dytiscidae), 2nd ed.* Springer, Cham, pp. 373–399.

De Mira-Mendes C.V., Costa R.N., Dias I.R., Carilo Filho L.M., Mariano R., Le Pendu Y. & Solé M. 2019: Effects of increasing temperature on predator-prey interaction between beetle larvae and tadpoles. — *Stud. Neotrop. Fauna Environ.* **54**: 163–168.

Frances D.N. & McCauley S.J. 2018: Warming drives higher rates of prey consumption and increases rates of intraguild predation. — *Oecologia* **187**: 585–596.

Fukuoka T., Kubo S., Ota M., Ohba S. & Yuma M. 2021: Feeding habits and prey selection of the diving beetle *Cybister brevis* (Coleoptera: Dytiscidae) larvae. — *Jpn. J. Environ. Entomol. Zool.* **32**: 1–7 [in Japanese, English abstract].

Fukuoka T., Tamura R., Yamasaki S. & Ohba S. 2023: Effects of different prey on larval growth in the diving beetle *Cybister sugillatus* Erichson, 1834 (Coleoptera: Dytiscidae). — *Aquat. Insects* **44**: 226–234.

Fukuoka T., Tamura R., Ohba S. & Yuma M. 2024: Different habitat use of two *Cybister* (Coleoptera: Dytiscidae) species larvae in a paddy field water system. — *Entomol. Sci.* **27**: e12595, 7 pp.

Hájek J., Hendrich L., Vyhnálek V. & Csabai Z. 2014: *Eretes* diving beetles (Coleoptera: Dytiscidae) in Central Europe – witnesses of climate change? — *Aquat. Insects* **36**: 267–271.

Hassall C. & Thompson D.J. 2008: The effects of environmental warming on Odonata: A review. — *Int. J. Odonatol.* **11**: 131–153.

Hickling R., Roy D.B., Hill J.K. & Thomas C.D. 2005: A northward shift of range margins in British Odonata. — *Global Change Biol.* **11**: 502–506.

Hickling R., Roy D.B., Hill J.K., Fox R. & Thomas C.D. 2006: The distributions of a wide range of taxonomic groups are expanding polewards. — *Global Change Biol.* **12**: 450–455.

Ichikawa N. 2008: The present condition and the preservation of pond insects lived in village. — *Jpn. J. Environ. Entomol. Zool.* **19**: 47–50 [in Japanese].

Juliano S.A. & Lawton J.H. 1990: The relationship between competition and morphology. II. Experiments on morphological patterns among co-occurring dytiscid beetles. — *J. Anim. Ecol.* **59**: 831–848.

Li F., Kwon Y., Bae M., Chung N., Kwon T. & Park Y. 2014: Potential impacts of global warming on the diversity and distribution of stream insects in South Korea. — *Conserv. Biol.* **28**: 498–508.

Mangiafico S. 2025: *rcompanion: Functions to Support Extension Education Program Evaluation.* (Version 2.5.0). URL: https://cran.r-project.org/package=rcompanion

McDaniel C.H., McHugh J.V. & Batzer D.P. 2017: Congeneric predaceous diving beetle species fail to segregate in a floodplain system: a case of amplified sympatry. — *Environ. Entomol.* **46**: 494–501.

Menéndez R. 2007: How are insects responding to global warming? — *Tijd. Entomol.* **150**: 355–365.

Ministry of the Environment of Japan 2015: *Red Data Book 2014: Threatened Wildlife of Japan, 5 Insecta.* Gyosei Corporation, Tokyo [in Japanese].

Ministry of the Environment of Japan 2020: *Red List of Japan.* URL: https://www.env.go.jp/press/107905.html (last accessed 14 Jan. 2024) [in Japanese].

Mitamura T., Hirasawa K. & Yoshii S. 2017: *The Handbook of Japanese Aquatic Insects. Vol. 1: Coleoptera.* Bun-ichi Sogo Shuppan, Tokyo [in Japanese].

Nagano K., Hiraiwa M.K., Ishiwaka N., Seko Y., Hashimoto K., Uchida T., Sánchez-Bayo F. & Hayasaka D. 2023: Global warming intensifies the interference competition by a poleward-expanding invader on a native dragonfly species. — *R. Soc. Open Sci.* **10**: 230449, 10 pp.

Nakajima J., Hayashi M., Ishida K., Kitano T. & Yoshitomi H. 2020: *Aquatic Coleoptera and Hemiptera of Japan.* Bun-ichi Sogo Shuppan, Tokyo [in Japanese].

Navara T., Kokavec I., Cíbik J., Lukáš J. & Chvojka P. 2020: *Adicella syriaca* (Trichoptera: Leptoceridae) in Slovakia – the northernmost record from Central Europe. Spreading of an aquatic insect in the period of climate change? — *Biologia* **75**: 2321–2326.

Nishihara S., Karube H. & Washitani I. 2006: Status and conservation of diving beetles inhabiting rice paddies. — *Jpn. J. Conserv. Ecol.* **11**: 143–157 [in Japanese, English abstract].

Nitzu E. 2022: *Eretes sticticus* (Coleoptera, Dytiscidae) – A new record for the Romanian fauna, and new data regarding the distribution of two rare species – *Acinopus ammophilus* (Coleoptera, Carabidae) and *Sphenoptera* (Chilostetha) *laportei* (Coleoptera, Buprestidae) in Dobrogea (Romania). — *Trav. Mus. Natn. Hist. Nat. Gr. Antipa* **65**: 55–61.

Ohba S. 2009: Feeding habits of the diving beetle larvae, *Cybister brevis* Aubé (Coleoptera: Dytiscidae) in Japanese wetlands. — *Appl. Entomol. Zool.* **44**: 447–453.

Ohba S. & Inatani Y. 2012: Feeding preferences of the endangered diving beetle *Cybister tripunctatus orientalis* Gschwendtner (Coleoptera: Dytiscidae). — *Psyche* **2012**: 1–3.

Ohba S. & Ogushi S. 2020: Larval feeding habits of an endangered diving beetle, *Cybister tripunctatus lateralis* (Coleoptera: Dytiscidae), in its natural habitat. — *Jpn. J. Environ. Entomol. Zool.* **31**: 95–100.

Ohba S., Fukui M., Terazono Y. & Takada S. 2020: Effects of temperature on life histories of three endangered Japanese diving beetle species. — *Entomol. Exp. Appl.* **168**: 808–816.

Ohba S., Terazono Y. & Takada S. 2022: Interspecific competition amongst three species of large-bodied diving beetles: Is the species with expanded distribution an active swimmer and a better forager? — *Hydrobiologia* **849**: 1149–1160.

Ohba S., Ogushi S., Goto N. & Watanabe R. 2023: The overwintering ecology of *Cybister tripunctatus lateralis*: Do subzero temperatures during winter suppress range expansion? — *Jpn. J. Environ. Entomol. Zool.* **34**: 101–107.

Ohba S., Suzuki T., Fukui M., Hirai S., Nakashima K., Bae Y. J. & Tojo K. 2025: Flight characteristics and phylogeography in three large-bodied diving beetle species: Evidence that the species with expanded distribution is an active flier. — *Biol. J. Linn. Soc.* **144**: blae017, 13 pp.

Ott J. 2010: Dragonflies and climatic change – Recent trends in Germany and Europe. — *BioRisk* **5**: 253–286.

Petrov P. & Fedorova D. 2013: *Cybister lateralimarginalis* in rather northern Russia. — *Latissimus* **33**: 7–8.

Pitcher K.A. & Yee D.A. 2014: Investigating habitat use, prey consumption, and dispersal response as potential coexistence mechanisms using morphologically similar species of predaceous diving beetles (Coleoptera: Dytiscidae). — *Ann. Entomol. Soc. Am.* **107**: 582–591.

R Core Team 2024: *A language and environment for statistical computing.* R foundation for statistical computing, Vienna, Austria. URL: https://www.R-project.org/

Robinet C. & Roques A. 2010: Direct impacts of recent climate warming on insect populations. — *Integr. Zool.* **5**: 132–142.

Saijo S. 2001: Seasonal prevalence and migration of aquatic insects in paddies and an irrigation pond in Shimane prefecture. — *Jpn. J. Ecol.* **51**: 1–11 [in Japanese, English abstract].

Sáinz-Bariáin M., Zamora-Munoz C., Soler J.J., Bonada N., Sáinz-Cantero C.E. & Alba-Tercedor J. 2016: Changes in Mediterranean high mountain Trichoptera communities after a 20-year period. — *Aquat. Sci.* **78**: 669–682.

Scheers K. 2014: On the increase of *Hydrovatus cuspidatus* (Kunze, 1818) and *Graptodytes bilineatus* (Sturm, 1835) (Coleoptera: Dytiscidae) in Belgium. — *Bulletin de La Société Royale Belge d'Entomologie/Bulletin van de Koninklijke Belgische Vereniging Voor Entomologie* **150**: 195–198.

Scheers K. 2017: *Hydrovatus clypealis* Sharp, 1876 expands its distribution to Belgium, with an update on the distribution of *H. cuspidatus* (Kunze, 1818) (Coleoptera: Dytiscidae). — *Bulletin de La Société Royale Belge d'Entomologie/Bulletin van de Koninklijke Belgische Vereniging Voor Entomologie* **153**: 198–202.

Scheirer C.J., Ray W.S. & Hare N. 1976: The analysis of ranked data derived from completely randomized factorial designs. — *Biometrics* **32**: 429–434.

Shah A.A., Dillon M.E., Hotaling S. & Woods H.A. 2020: High elevation insect communities face shifting ecological and evolutionary landscapes. — *Curr. Opin. Insect Sci.* **41**: 1–6.

Shimono M. 2015: Recent trends of *Cybister tripunctatus lateralis* in Yamaguchi Prefecture. — *Yamaguchi No Mushi* **14**: 84–90 [in Japanese].

Thomas J. 2009: *Cybister lateralimarginalis* (De Geer) (Dytiscidae) discovered in Britain for the first time since the 19th century. *The Coleopterist* **18**: 145–147.

Twardochleb L.A., Treakle T.C. & Zarnetske P.L. 2020: Foraging strategy mediates ectotherm predator–prey responses to climate warming. — *Ecology* **101**: e03146.

Venables W.N. & Ripley B.D. 2002: *Modern applied statistics with S. Fourth edn.* Springer, New York.

Watanabe K. 2021: Current state about establishment of *Cybister tripunctatus lateralis* (Fabricius, 1798) (Coleoptera: Dytiscidae) in Ishikawa Prefecture, Japan. — *SAYABANE New Series* **41**: 1–5 [in Japanese].

Watanabe K., Inoda T., Suda M. & Yoshida W. 2021: Larval rearing methods for two endangered species of diving beetle, *Cybister chinensis* Motschulsky, 1854 and *Cybister lewisianus* Sharp, 1873 (Coleoptera: Dytiscidae), using laboratory-bred food prey. — *Coleopt. Bull.* **75**: 440–444.

Watanabe R., Ohba S. & Sagawa S. 2024: Coexistence mechanism of sympatric predaceous diving beetle larvae. — *Ecology* **105**: e4267, 5 pp.

Yee D.A. 2010: Behavior and aquatic plants as factors affecting predation by three species of larval predaceous diving beetles (Coleoptera: Dytiscidae). — *Hydrobiologia* **637**: 33–43.

Yee D.A., O'Regan S.M., Wohlfahrt B. & Vamosi S.M. 2013: Variation in prey-specific consumption rates and patterns of field co-occurrence for two larval predaceous diving beetles. — *Hydrobiologia* **718**: 17–25.

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Fig. S1. Scene of a container in an experiment to quantify behavior.

Fig. S2. Scene of a container in an experiment on the time taken to reach food. The food was placed on the surface of the water 5 cm away from the larval head (red arrow).

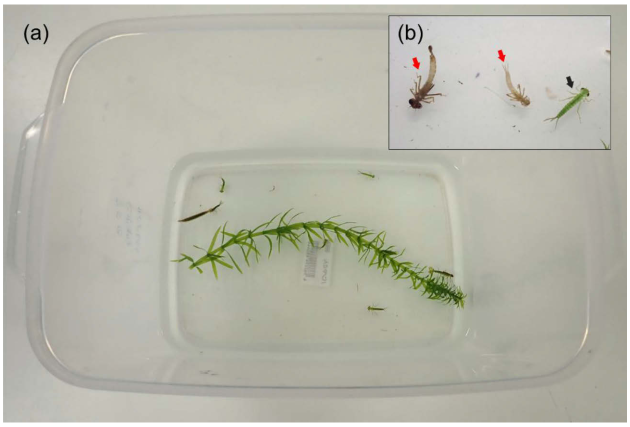

Fig. S3. Scene of the container in an experiment on the number of prey animals consumed (a). Prey animals predated by the larvae lose body color (red arrow: predated, black arrow: alive), which was used to determine the presence or absence of consumption (b).

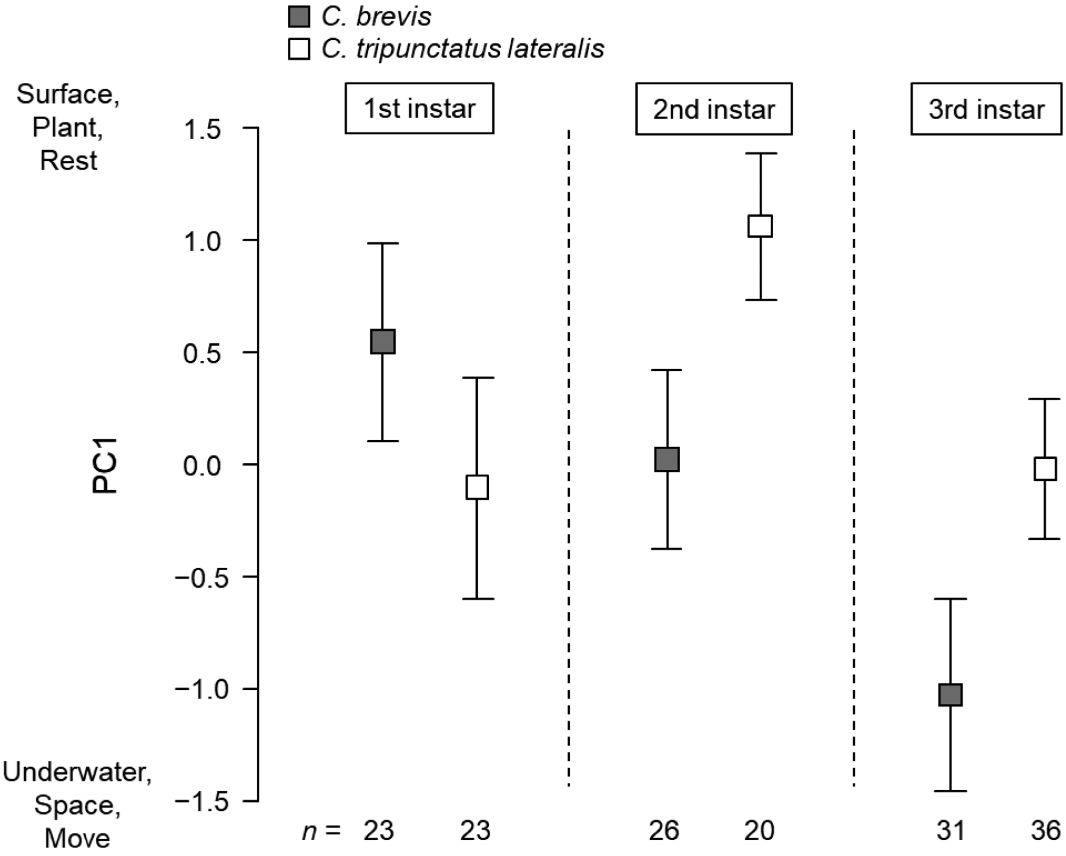

Fig. S4. PC1 scores (mean ± standard error) of the two species. The interpretation of PC1 is that larger positive values indicate surface, plant, and rest behaviors, while larger negative values indicate underwater, space, and move behaviors. Figures below the plots indicate the sample size.

Table S1. Results of the principal component analysis (PCA) for larval behavior.

	PC1	PC2
Position		
Surface	**0.385**	**−0.529**
Underwater	**−0.385**	**0.529**
Location		
Plant	**0.398**	**0.467**
Space	**−0.398**	**−0.467**
Activity		
Move	**−0.440**	−0.041
Rest	**0.440**	0.041
Proportion of variance	0.766	0.174
Cumulative variance	0.766	0.940
Interpretation	+ (Surface, Plant, Rest) vs. − (Underwater, Space, Move)	+ (Underwater, Plant) vs. − (Surface, Space)

Values >0.35 are shown in bold, indicating strong loadings on each principal component.

Table S2. Results of the generalized linear mixed model (GLM) fitted to food discovery rate.

	Estimate	SE	z	P-value
(Intercept)	20.57	4179	0.005	0.996
C. tripunctatus lateralis [a]	−18.55	4179	−0.004	0.996
2nd instar [b]	−18.67	4179	−0.004	0.996
3rd instar [c]	<−0.0001	5760	0.000	1.000
C. tripunctatus lateralis: 2nd instar	19.49	4179	0.005	0.996
C. tripunctatus lateralis: 3rd instar	18.55	6535	0.003	0.998

[a] The coefficient indicates the relative effect of *C. tripunctatus lateralis* compared with *C. brevis*. [b] The coefficient indicates the relative effect of second instar compared with first instar. [c] The coefficient indicates the relative effect of third instar compared with first instar.