Chlorantraniliprole application differentially affects adult emergence of *Sympetrum* dragonflies in rice paddy fields

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Abstract

Rice paddy fields are important habitat for many dragonfly species. In Japan, populations of dragonflies inhabiting rice paddies, in particular *Sympetrum* (Odonata: Libellulidae), have decreased greatly in the last few decades. A major cause of the decline has been suggested to be the use of systemic insecticides (e.g., phenylpyrazole and neonicotinoid) in nursery boxes of rice seedlings. In this study, we examined the effects of chlorantraniliprole (CAP), a novel anthranilic diamide insecticide, on adult emergence of *Sympetrum* dragonflies in ten rice paddy fields by counting their exuviae remaining on the rice plants as an abundance index. Our results suggest that CAP is a potential factor that reduced the emergence rate of *S. infuscatum* but not of *S. frequens*. This difference may be due to differential sensitivity to CAP, different lengths of the nymphal stage, or different effects of bottom-up controls via reduction of prey organisms that are highly sensitive to CAP.

Keywords Anthranilic diamide · Dragonfly · Exuviae · Odonata · *Sympetrum frequens* · *Sympetrum infuscatum*

Introduction

Insecticide use in agriculture is of concern as a major cause of insect declines worldwide (Sánchez-Bayo and Wyckhuys 2019; Forister et al. 2019; Cardoso et al. 2020; Wagner 2020). In Japan, rice paddy fields are utilized as wetland-like habitats by many species; they occupy approximately 24 000 km² (about half of the total cultivated area) (Ministry of Agriculture, Forestry and Fisheries 2019), whereas the area of natural wetlands has decreased greatly over the past century from 2110 to 820 km² (Geospatial Information Authority of Japan, 2000). These rice fields and their irrigation systems serve as main breeding

sites for many dragonfly (Odonata) species; as many as 31 species of a total of approximately 200 resident species use rice paddies (Uéda 1998). However, many Japanese dragonfly species have been threatened with extinction in recent decades (Kadoya et al. 2009). The application of certain types of systemic insecticides to nursery boxes of rice seedlings (i.e., the phenylpyrazole fipronil and the neonicotinoid imidacloprid) is suspected as one of the main causes of the sharp population declines of *Sympetrum frequens* (Odonata: Libellulidae)—one of the most common dragonfly species in Japan—in the 1990s (Nakanishi et al. 2018, 2020). By the 2010s, the population density of *S. frequens* had not recovered to pre-1990s levels, even though the use of fipronil and imidacloprid has decreased (Nakanishi et al. 2018, 2020). Other types of insecticides (e.g., cartap and chlorantraniliprole) are also suspected of limiting the population growth of *S. frequens* in addition to other agronomic factors, such as midsummer drainage and crop rotation (Nakanishi et al. 2020). Because dragonflies play an important role as common intermediate predators in both aquatic (e.g., rice paddies) and terrestrial ecosystems (Corbet 1999), it is important to detect current agronomic factors reducing their population growth for biodiversity conservation in Japanese rural areas.

A recently introduced chemical, chlorantraniliprole (CAP; Rynaxypyr®), an anthranilic diamide insecticide, has been commonly used as a nursery box treatment for

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rice cultivation since the 2010s in Japan (Nakanishi et al. 2020). CAP has a different mode of action from neurotoxic insecticides such as pyrethroids and neonicotinoids: it acts on insect ryanodine receptors and causes excessive release of internal calcium, resulting in death by inhibiting feeding and paralyzing muscles (Lahm et al. 2007). Because CAP has high selective toxicity against insects over mammals, it has been widely used for pest control in various crops and is expected to be helpful in integrated pest management (Lahm et al. 2007, 2009). Although CAP has little adverse effect on dragonflies (adult emergence) (Kasai et al. 2016; Miyai et al. 2016; Hashimoto et al. 2020), it has higher toxicity than neonicotinoids and phenylpyrazoles to other aquatic invertebrates such as water fleas (EFSA 2013; EPA 2019). Nevertheless, little is known about its effects on aquatic organisms in production of rice paddies.

Most previous studies used small-scale lysimeters or rice paddy mesocosms to examine the effects of pesticides on aquatic communities (e.g., Hashimoto et al. 2020; Hayasaka et al. 2012; Jinguji et al. 2013; Miles et al. 2017); studies using commercial rice paddies are rare (Suhling et al. 2000). Although some observational studies of the effects of pesticides have been conducted in rice paddies, agronomic factors other than target pesticides were not properly controlled (e.g., Baba et al. 2019; Katayama et al. 2019; Wilson et al. 2007). In addition, although up to 31 dragonfly species inhabit rice paddies in Japan and several species often coexist (Uéda 1998), little is known about species differences in sensitivity to pesticides.

Here, we examined the effects of CAP application in rice paddies on the adult emergence of dragonflies of the genus *Sympetrum*, one of the most common genera inhabiting rice paddy fields in Japan (Uéda 1998). We conducted a field survey in commercial rice paddies with and without CAP. As an index of abundance, we recorded exuviae, which have been used before as environmental indicators in rice paddy ecosystems (Suhling et al. 2000; Baba et al. 2019; Katayama et al. 2019); we compared numbers between treatments, because exuviae are proof of success in nymphal development and adult emergence at a site, and because they provide the overall numbers of dragonflies that have emerged, being less affected by survey time compared with adult numbers (Raebel et al. 2010). We analyzed the effects of CAP on the emergence of each *Sympetrum* species, taking account of other environmental factors such as water depth, and thereby examined interspecific differences in sensitivity to CAP. We hypothesized that CAP application would decrease the emergence of *Sympetrum* species in rice paddies, as it does in many other aquatic invertebrates (EFSA 2013; EPA 2019).

Methods

Species investigated

Sympetrum is a common genus in rice paddy fields throughout Japan (Uéda 1998). In the study area, *S. frequens* and *S. infuscatum* are numerically dominant. Both species have a univoltine life cycle. They lay eggs in drained paddies in autumn. The main reproductive season of *S. infuscatum* is August to September (Watanabe et al. 2004), whereas that of *S. frequens* is September to November (Uéda 1988). The eggs overwinter and hatch the following spring (around April), soon after the start of irrigation. The nymphs grow while feeding on other aquatic organisms, such as sludge worms, microcrustaceans, and midge and mosquito larvae, and they emerge as adults in June and July (Inoue and Tani 2010). The nymphal period of *S. infuscatum* is about 2 weeks longer than that of *S. frequens*, and *S. infuscatum* nymphs grow larger than *S. frequens* nymphs (Jinguji and Tsuyuzaki 2009).

Experimental design

To examine the effect of CAP application on adult emergence, we used ten adjacent commercial rice paddy fields in Hakui City, Ishikawa Prefecture, Japan (36.920317 N, 136.784380 E; Fig. 1). The study site was located in a paddy-dominated landscape of Ouchi Plain. The ten paddy fields were situated in two blocks (A and B) separated by a road, with opposite water flow directions. Individual fields were approximately 0.3 ha. We assigned treatments in consultation with the farmer so as to create identical conditions apart from the insecticide application; six fields were treated with CAP and the other four fields were used as controls. The fields grew rice (*Oryza sativa* L. ‘Koshihikari’) under the conventional management regime in the region except for the application of CAP to rice seedlings. In this region, CAP has been used during nursery or rice planting since 2015, including in our study fields. All study fields had been cultivated under the same regime with equal amounts of pesticides (including CAP) and fertilizers in the previous year. The CAP-treated fields were treated with a commercial agrochemical product, Dr. Oryze®-Ferterra® (0.75% granular CAP and 24% granular probenazole, Hokko Chemical Industry Co., Ltd., Tokyo, Japan), at 10 kg ha⁻¹ (recommended dose) just to the side of the rice seedlings at the time of transplantation (18–20 May 2019). The fields were flooded continuously from 7–8 May to 22–25 June, and then temporarily drained (midsummer drainage). After the midsummer drainage began, they were irrigated intermittently every 4 days or so until 30 August.

Fig. 1 Plan of experimental paddy fields. Chlorantraniliprole (CAP) was applied to nursery boxes of rice seedlings later planted in the six CAP fields, but no insecticide was used in the four control fields. Cross marks indicate fields not used in the study

Water was supplied to each field from an inlet (via a spigot valve) and drained from an outlet (Fig. 1), so the insecticide was unlikely to contaminate other fields through the irrigation system. Since the agrochemical contained not only CAP but also probenazole, our experimental design could not isolate the effect of CAP. However, a previous study showed no statistically significant adverse effect of probenazole on *S. frequens* (Shimada et al. 2004), and the HC₅ (hazardous concentration at which 5% of species would be affected) for aquatic ecosystems on the basis of the species sensitivity distribution of probenazole is predicted to be approximately 290 times greater than that of CAP (Nagai 2016).

Dragonfly sampling and environmental measurement

We surveyed dragonfly exuviae about every 10 days from 7 June to 30 July 2019, six times in total. Prior to the survey, we established three plots [20 m × 1 m (consisting of ca. 100 × 3 rows of rice plants)] alongside levees in each field (Fig. 1). At each plot, we collected exuviae from rice stems in three rows of plants according to the sampling method of Baba et al. (2019). At the respective plot, we also collected exuviae from weeds grown among rice plants. Samples were

identified to species level under a stereomicroscope (LZ-LED-T, Kenis, Osaka, Japan) in the laboratory using the guide by Inoue and Tani (2010).

From 7 to 26 June (three measurements), prior to the start of midsummer drainage, we measured water depth in each field, because we could not control water depth among the fields. At each survey, we measured water depth (from the paddy sediment surface to the water surface) at the levee-side center of each plot using a fiberglass folding rule (Shinwa Rules Co., Ltd., Niigata, Japan). For each field, we calculated the arithmetic means of the three water depth measurements across the pre-midsummer drainage period.

Statistical analyses

To examine the effect of CAP on adult emergence, we conducted a multiple regression analysis using a generalized linear model (GLM) (Nelder and Wedderburn 1972; McCullagh and Nelder 1989). We mainly interpreted the result based on the GLM rather than a simple two-group comparison (e.g., Wilcoxon rank sum test). This is because GLM analysis can consider the effect of CAP by statistically controlling for the effects of other environmental factors. In Wilcoxon rank sum test, the severe limitation of sample size

may not assure effective randomization (i.e., risk of random confounding; Suzuki et al. 2020) and may not provide sufficient statistical power to detect the effect of CAP under other large sources of variation (e.g., block and water depth) in the paddy fields (see Results). Therefore, we show the result of GLM analysis in the main text and provide the results of Wilcoxon rank sum test in the supplementary material.

Because the count data were over-dispersed (see Supplementary code 2.1), we applied a negative binomial distribution (log-link function) to the GLM (McCullagh and Nelder 1989; Crawley 2013). We developed the following model, which takes into account the effects of blocks and water depth:

$$\log(\lambda_i) = \alpha + \sum_{j=1}^J \beta_j x_{j,i} + \varepsilon_i \quad (1)$$

$$y_i \sim \text{Negative binomial}(\lambda_i, \theta) \quad (2)$$

where λ_i and y_i represent the expected and counted total emergence numbers in the i th field. $x_{j,i}$ represents j th explanatory variable in the i th field. β_j is the coefficient for the j th explanatory variable. α represents an intercept and ε_i is an error term in i th field. θ denote the shape parameter of the negative binomial distribution. We used block (categorical variable; block A as reference category), CAP treatment (dummy variable; 0 for control, 1 for CAP treatments), species (categorical variable; *S. frequens* as reference category), mean water depth (means of the three measurements), and interaction of CAP $\times$ species for explanatory variables.

All analyses were conducted in R v. 3.6.1 software (R Core Team 2019). For the GLM, we used the *glm.nb* function in the MASS package (Venables and Ripley 2002). All data used in the analyses and R code are available in online resources 1 and 2, respectively.

Results

Adult emergence rates over time

In total, we collected 475 exuviae of *Sympetrum* dragonflies: 239 *S. frequens*, 233 *S. infuscatum*, and 3 fragmented and thus unidentifiable to species level. Only one exuvia (*Orthetrum albistylum speciosum*) of the other dragonfly species was observed other than *Sympetrum* in the study fields. We observed adult emergence of *S. frequens* from 18 June to 18 July and of *S. infuscatum* from 26 June to 3 July (Fig. 2). Numbers fluctuated similarly between species and between treatments; numbers peaked on 26 June, although *S. frequens* began to emerge around 1 week earlier than *S. infuscatum*.

Effects of chlorantraniliprole and other environmental factors

The variations in emergence among fields in each treatment, particularly in the control, were wide (Fig. 3). GLM analysis indicated a significant block effect ($P < 0.001$; Table 1). Although the effect of CAP treatment was not significant ($P = 0.416$), the CAP $\times$ species interaction significantly affected emergence ($P = 0.023$). In *S. infuscatum*, emergence at the peak was 88% less in the CAP treatment relative to that in the CAP-free control. By contrast, in *S. frequens*, emergence did not differ between the two treatments. The effect of mean water depth was not significant ($P = 0.815$).

Wilcoxon rank sum test gave no statistically significant difference in the emergence numbers between treatments with and without CAP in either *Sympetrum* species (see Supplementary code 2.4). Factors unrelated to CAP could have caused the large variances. In this situation, the statistical power of rank-based non-parametric tests for two-group comparison was severely reduced.

Fig. 2 Temporal changes in mean numbers of emerged individuals of indicated species per field with or without chlorantraniliprole (CAP) treatment from 7 June to 30 July 2019

Fig. 3 Numbers of emerged individuals of indicated species in the chlorantraniliprole (CAP) and control treatments

Table 1 Results of negative binomial generalized linear model examining the effect of chlorantraniliprole (CAP) and other environmental factors on the adult emergence of *Sympetrum frequens* and *S. infuscatum*

Variable	Estimated coefficient	SE	<i>P</i> value	
(Intercept)	3.837	1.257		
Block [†]	−2.503	0.472	<0.001	**
CAP	0.418	0.513	0.416	
Species [‡]	−0.075	0.546	0.891	
Water depth (cm)	0.039	0.166	0.815	
CAP×species	−1.676	0.739	0.023	*

[†] Block A as reference

[‡] *Sympetrum frequens* as reference

***P* < 0.001, **P* < 0.05 (Wald test)

Discussion

We show that CAP is a potential factor that has adverse effects on the emergence of *S. infuscatum* but not of *S. frequens* (Table 1; Fig. 3). Our result contrast with preceding mesocosm and field studies. Previous studies using mesocosms or field observations showed that CAP had little adverse effect on the adult emergence of dragonflies, including *Sympetrum* (Miyai et al. 2016), *Crocothemis*, and *Orthetrum* (Kasai et al. 2016) in rice paddies (Kasai et al. 2016; Miyai et al. 2016). In a field study, Huynh et al. (2021) found no statistically significant difference in the adult emergence of *Sympetrum* dragonflies between conventional (with synthetic pesticides and fertilizers, including CAP) and natural rice paddies (without synthetic pesticides or fertilizers of any kind). Because these previous studies were not designed to elucidate the effects of CAP itself on the adult emergence of *Sympetrum* dragonflies,

agronomic factors other than CAP were not sufficiently controlled (Miyai et al. 2016; Huynh et al. 2021).

There are three potential mechanisms for differential effects of CAP application on the two *Sympetrum* species and these mechanisms are not mutually exclusive. First, the two species may have different sensitivities to CAP, although there are no published data on laboratory toxicity testing on the two species. Second, a difference in life history during their nymphal periods may affect their mortality under CAP exposure. Jinguji and Tsuyuzaki (2009) reported that the mean nymphal period of *S. infuscatum* was about 2 weeks longer than that of *S. frequens*, and the body size of the last-instar nymphs of *S. infuscatum* was larger than that of *S. frequens*. Our result is consistent with this difference in their nymphal periods: the start of adult emergence of *S. infuscatum* was later than that of *S. frequens* (Fig. 1). Therefore, the nymphs of *S. infuscatum* may have been exposed longer to CAP than *S. frequens* before emergence. Third, the results of previous experimental studies of the potential prey organisms of the dragonflies suggest that the effects of CAP on adult emergence may occur indirectly through bottom-up control in the food chain. This control is expected to more strongly affect *S. infuscatum*, with its longer nymphal period. Saito et al. (2014) reported that the nymphal growth of *S. frequens* was poorer in paddies with CAP application than without. This difference may have resulted from reduced abundance of prey organisms in rice paddies with CAP application, owing to high acute toxicity to invertebrate prey such as water fleas (e.g., Cui et al. 2017; Lavtižar et al. 2015) and midges (e.g., Maloney et al. 2019; Rodrigues et al. 2015). Likewise, Hashimoto et al. (2020) showed negative effects of CAP on the abundance of some invertebrates such as sludge worms and microcrustaceans in a paddy mesocosm experiment in which CAP was applied at the same dose as here; they reported that the maximum concentration of CAP in paddy water reached 5–15 µg/L within a few days after transplantation of rice seedlings. If the environmental fate

of CAP in our study was similar to the results of Hashimoto et al. (2020), CAP application may have resulted in reduced abundance of microcrustacean prey, as the 48-h EC_{50} of the water flea, *Daphnia magna* is 7.1–16.6 $\mu\text{g/L}$ (EPA 2019). This hypothesis needs to be tested in future studies.

As we used commercial rice paddies, some limitations must be resolved in evaluating the effects of CAP. First, there was a large variation in the numbers of exuviae among fields within treatments (Fig. 3), and block had statistically significant effects on numbers (Table 1). The four fields with the highest numbers of individuals of both species belonged to block A (Fig. 3). In GLM, the mean water depth had no statistically significant effects on dragonfly emergence. Furthermore, there was no significant difference in mean water depth between blocks. Nevertheless, the fields with many emergence numbers tended to have a greater mean water depth (see Supplementary code 2.5). Therefore, the environmental conditions such as the degree of drainage may not have been effectively randomized for the treatment and block owing to the small sample size. This variation in emergence numbers among fields within the treatment may have been caused by differences in the initial abundance of the dragonflies (i.e., numbers of eggs), which could not be readily identified before the assignment of treatments here. Future work should examine adult oviposition in each field in the year before exuviae are surveyed. Second, monitoring of environmental concentrations and the fate of CAP in the fields are needed in order to quantify the effects of the insecticide on the dragonflies. Because CAP has high persistence in paddy soil (Hashimoto et al. 2020), emergence numbers in the control treatment could have been driven down by CAP residues from previous years. Our results thus could have underestimated the effects of CAP on the dragonflies. Third, because CAP can also affect species compositions of various aquatic organisms, including prey, predators, and competitors with *Sympetrum* dragonflies (Kasai et al. 2016; Hashimoto et al. 2020), we need to clarify the biological interactions involving *Sympetrum* dragonflies (e.g., observation of predator–prey interactions) as a next step in assessing the effects in rice paddies of CAP application.

In conclusion, our results suggest that CAP is a potential factor that reduces the adult emergence of *S. infuscatum*, but not of *S. frequens*. Such interspecific difference in susceptibility to CAP may be caused by a difference in sensitivity to the insecticide or a difference in the period of the nymphal stage, as nymphs consume aquatic invertebrates that are highly sensitive to CAP. As neonicotinoid insecticides can have adverse indirect effects on insectivorous birds through the food web (Hallmann et al. 2014), CAP also may harm terrestrial ecosystems through a decrease in populations of dragonflies, which are an important food source for insectivorous birds (Corbet 1999). For biodiversity conservation in Japanese rural areas, we need to quantitatively evaluate the effects of insecticides

and to seek an effective approach in the use of insecticides with minimum adverse effect on dragonflies.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10333-021-00880-5>.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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