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Do Agrochemical-Free Paddy Fields Serve as Refuge Habitats for Odonata?

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Abstract: Agrochemical-free rice farming has attracted interest for restoring paddy field biodiversity and producing safe food. Odonata are commonly used as a biodiversity indicator in these low-input farms. However, the effect of agrochemical-free rice farming on odonate diversity has rarely been assessed over the entire emergence period of these insects. We investigated whether different farming practices, such as conventional or natural (agrochemical-and fertilizer-free) cultivation, and associated water management strategies affect the emergence rates of Odonata in paddy field landscapes in central Japan. Weekly exuviae sampling in 2017 and 2019 suggested that odonate assemblages differed between conventional and natural paddy fields, with a higher number of taxa emerging from natural paddy fields. Contrary to expectations, conventional paddy fields had equivalent or higher emergence rates of all Odonata and two numerically dominant *Sympetrum* species. Peak emergence periods for numerically dominant taxa differed between the farming types, with the emergence of three *Sympetrum* species peaking in late June in conventional paddy fields and that of *S. frequens* peaking in early to mid-July in natural paddy fields. Our findings suggest that both conventional and natural paddy fields are important habitats for Odonata in Japan.

Keywords: wildlife-friendly farming; environmentally friendly farming; organic farming; rice field; exuviae; dragonfly; damselfly; *Sympetrum*

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1. Introduction

Over the past century, land-use change has led to a 61% decline in natural wetlands in Japan [1]. Among natural wetlands, floodplains have undergone extensive conversion into farmland, typically to rice (*Oryza sativa*) paddy fields. Although paddy fields were established to meet rising food demands, these anthropogenic wetlands also serve as refuge habitats for aquatic and semi-aquatic wildlife including waterfowl, amphibians, fish, spiders, and insects [2–9].

Increased attention has been given to the social–ecological restoration of Satoyama landscapes in Japan, which comprise paddy fields, cropland, irrigation ponds, streams and ditches, secondary forests, and grasslands around human settlements in rural areas [10]. Wildlife-friendly farming is one tool used to meet restoration goals. Examples of wildlife-friendly rice farming practices include agrochemical-free farming (i.e., natural or organic farming), agrochemical-reduction farming, and winter flooding practices [10].

To evaluate the effectiveness of wildlife-friendly rice farming in Japan, various indicator taxa have been proposed including plants, spiders, insects, frogs, fish, crustaceans, and wading birds [11,12]. Among insects, Odonata have been widely used to assess the negative impacts of insecticides [13–16]. Consequently, several studies have evaluated the effectiveness of wildlife-friendly farming using Odonata as an indicator group [17–23]. However, those studies have typically been conducted over limited sampling periods [17,20,23], with no or few replicates at the field level [18], or focused on a single odonate taxon that might not represent the overall assemblage [19]. Little is known about the effects of different farming practices on the composition of Odonata assemblages throughout their emergence periods.

Studies have also typically used organic paddy fields as representative of "low-input" agriculture. Although agrochemicals are not used in organic farming, some organic fertilizers that are applied to paddy fields after rice transplantation have been shown to have detrimental effects on aquatic macroinvertebrates due to the resulting low concentration of dissolved oxygen in water [14,24]. Furthermore, herbicides or heavy metals are sometimes detected at high concentrations in animal manure fertilizers [25,26]. Therefore, in this study, we used agrochemical- and fertilizer-free natural farms as low-input representatives.

Here, we investigated the effects of natural rice farming on Odonata assemblages over their entire emergence periods by comparing emergence rates between natural and conventional paddy fields. Because numerous small-scale microcosm or laboratory experiments have shown that Odonata are generally susceptible to synthetic chemical insecticides [15,16], we predicted that the emergence rates would be higher in natural paddy fields than in conventional paddy fields. We also hypothesized that the peak emergence periods would differ between natural and conventional paddy fields due to the different rice-transplantation periods and associated water management practices between the two farming practices (see Section 2.2).

2. Methods

2.1. Study Sites

The study sites were located on or around the Ouchi Plain in Hakui and Houdatsushimizu (hereafter, the Hakui–Houdatsushimizu area), Ishikawa Prefecture, in central Japan. Ouchi Lagoon west of Ouchi Plain has a history of land reclamation that dates back to 1574 [27]. From 1948 to 1968, most of Ouchi Lagoon (374 of 456 ha) was converted into rice paddies to meet the increasing demand for food and to revitalize the rural economy. The remaining 82 ha were retained as irrigation and drainage watercourses with concrete bank protection on both sides. Owing to habitat loss and degradation, various native species are believed to have disappeared from Ouchi Lagoon, which in turn, facilitated invasion by non-native species such as largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), common carp (*Cyprinus carpio*), and red swamp crayfish (*Procambarus clarkii*). Therefore, paddy fields in the former Ouchi Lagoon serve as refuge habitats for various aquatic and semi-aquatic wildlife that once inhabited the natural floodplain wetland.

However, the development and widespread use of agrochemicals in recent decades have led to dramatic declines in farmland biodiversity in this area. To restore degraded farmland and revitalize depopulated rural communities while producing safe food, JA (Japan Agricultural Cooperatives) Hakui and the Hakui City Government have been promoting natural farming in rice agriculture since 2010. Although rice yields are generally lower in natural farming relative to organic farming, the implementation of natural farming is expected to produce safe food and restore paddy field biodiversity.

We conducted field surveys in the Hakui–Houdatsushimizu area in 2017 and 2019 (Figure 1). In both years, we adopted a paired design—one conventional paddy field and one natural paddy field were arbitrarily chosen from each of eight regions (blocks) for a total of 16 paddy fields (Figure 1). Within a region, paddy fields were similar in terms of surface area and were located in close proximity to one another (<2 km). The overall mean (±SD) surface area of conventional paddy fields was 0.18 ha ± 0.10 and that of natural paddy fields was 0.19 ha ± 0.11. For both field types, we selected paddy fields that grew the cultivar "Koshihikari," a major rice variety in the area. For natural paddy fields, we selected paddy fields that were in at least the second (2017 survey) or fourth year (2019 survey) of natural farming to minimize the residual effects of agrochemicals from past conventional farming.

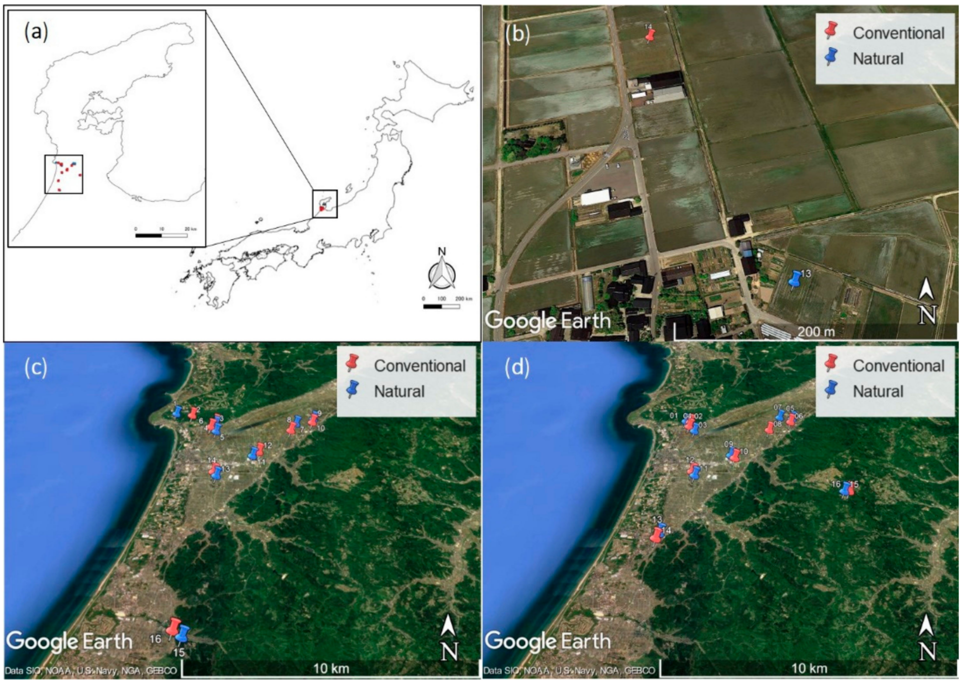

Figure 1. (**a**) Map of the study location in the Hakui–Houdatsushimizu area on the Noto Peninsula, central Japan. (**b**) Example of a pair of conventional and natural paddy fields, and the sampling sites in (**c**) 2017 and (**d**) 2019. Map sources: (**a**) Geospatial Information Authority of Japan; (**b**–**d**) Google Earth (www.earth.google.com).

2.2. Farming Calendar for Conventional and Natural Practices

In the Hakui–Houdatsushimizu area, rice transplantation in conventional paddy fields generally takes place between late April and mid-May; rice is then harvested in early September of the same year. In conventional farming, rice plants are cultivated using synthetic chemical pesticides, such as insecticides, fungicides, and herbicides (Appendix A, Table A1). Depending on the type, pesticides are often applied several times before and after rice transplantation. Nursery box insecticides (Ferterra (chlorantraniliprole)) that are absorbed by rice seedlings during rice transplantation (late April–mid-May) and unmanned helicopter application (late July and early August) are the two common forms of pesticide application. In addition to pesticides, chemical fertilizers are generally applied to paddy fields one to three times per farming season (Appendix A, Table A1). To impede diversification of rice plant tillers and facilitate rice harvest using a combine harvester, water is usually drained from the paddy field for 5–25 days beginning in early to mid-June (called midseason drainage). Following midseason drainage, on-and-off irrigation, in which water is added once the paddy has become dry, is generally practiced until mid-August. Panicle initiation in conventional paddy fields usually takes place in late July or early August.

Rice transplantation in natural paddy fields generally takes place in early to mid-June, approximately 1 month later than in conventional paddy fields. Rice is harvested in late September of the same year. In natural farming, rice plants are cultivated without using synthetic chemical pesticides or chemical or organic fertilizers. Generally, rice straws are chipped and left on the paddy field over winter following rice threshing in late September. The following spring (typically March–April), dried rice straws are plowed into the soil. Generally, natural paddy fields are filled with irrigated water during the cultivation period until mid to late August, after which water is drained, and the rice is harvested. However, owing to water supply limitations, some farmers practice on-and-off irrigation during the summer. Weeds are removed using machinery once to several times between mid-June and mid-July, prior to panicle initiation. Panicle initiation typically occurs in mid-August in natural paddy fields, approximately two weeks later than in conventional paddy fields.

2.3. Life Histories of Representative Odonata

There are several main species of damselflies (Zygoptera) and dragonflies (Anisoptera) in the paddy-field landscapes in the Hakui–Houdatsushimizu area. The main Zygoptera species is *Ischnura asiatica* (Coenagrionidae), whereas the dominant Anisoptera, numerically speaking, are *Sympetrum frequens*, *S. infuscatum*, *S. darwinianum*, *Pantala flavescens*, and *Orthetrum albistylum* (all Libellulidae).

Ischnura asiatica has a bivoltine or multivoltine life cycle [28]. Adult *I. asiatica* lay eggs from May to November on aquatic plants near the water surface in various lentic waters in plain and mountain areas, including paddy fields. The egg stage lasts 1 to 3 weeks, whereas the larval stage lasts 1.5 to 7 months; this species is known to overwinter as larvae.

The three *Sympetrum* species (*S. darwinianum*, *S. infuscatum*, and *S. frequens*) have univoltine life cycles [28]. Adult *S. frequens* differ in their habitat use and oviposition behaviors from *S. darwinianum* and *S. infuscatum* [29]. In autumn, female *S. frequens* lay eggs in wet mud by protruding their abdomens, whereas female *S. darwinianum* and *S. infuscatum* drop eggs from the air. After overwintering as eggs, *Sympetrum* eggs hatch in early spring when the paddy fields are inundated with water. The larval stage of the three *Sympetrum* species lasts 3 to 5 months. In the study area, the three *Sympetrum* species start to emerge from mid-June, when most conventional paddy fields start to drain water for midseason drainage (Appendix A, Figure A1). After emergence, adult *S. frequens* migrate to distant highland areas during summer, whereas adult *S. darwinianum* and *S. infuscatum* move to nearby woodland areas. In autumn, adult *Sympetrum* return to the paddy fields and other wetland habitats for oviposition.

Pantala flavescens is a migratory species with a multivoltine life cycle [28]. A recent stable isotope study using stable hydrogen ($\delta\,^2$H) revealed that the majority of individuals found from April to November in southern, western, central, and northern Japan are migrants from continental Asia with broad geographic origins [30]. When adults arrive in Japan, they lay eggs in inundated paddy fields and open wetland habitats. The egg stage lasts 3 to 7 days, followed by a larval period of 1 to 2 months. *Pantala flavescens* can generally complete its life cycle within 1.5 months. Later in the season (October and November), locally reproduced individuals of Japanese origin have also been identified [30]. In most regions, *P. flavescens* is believed to die off and not overwinter, except in subtropical Ishigaki Island in southern Japan [28,29].

Orthetrum albistylum also has a multivoltine life cycle [28]. Adult *O. albistylum* lay eggs from mid-March to mid-December in various lentic waters, including paddy fields. The egg stage lasts 5 to 10 days, followed by a larval period of 2 to 8 months. This species overwinters as larvae.

2.4. Preliminary Survey (2017)

We performed a preliminary survey to identify odonate emergence periods in conventional and natural paddy fields. From June 17 to August 5 of 2017, we collected exuviae once a week from 16 paddy fields (8 weeks of sampling in total) (Appendix A, Figure A2). In each paddy field, we randomly chose 10 consecutive rice plants over three rows (30 rice plants) from two long sides and the outlet side, respectively, for a total of 90 rice plants (10 rice plants $\times$ 3 rows $\times$ 3 sides). Odonata exuviae were placed in wide mouth jars and transported back to the laboratory. Subsequently, we measured the water depth at three locations per side for a total of nine locations and calculated the mean depth as their average. In the laboratory, we air-dried Odonata exuviae and identified each specimen to the lowest taxonomic unit (to the species level) using a binocular microscope and identification keys [28,29,31].

2.5. Main Survey (2019)

In 2019, we scaled up the spatial extent of sampling from the preliminary work done in 2017. From June 11 to August 20 of 2019, we performed weekly exuviae sampling in 16 paddy fields (11 weeks in total). Between 2017 and 2019, some farmers shifted their farming practices, primarily from natural to conventional. As a result, seven of the 16 paddy fields surveyed in 2017 were retained in the study, and nine were shifted to new locations.

In each paddy field, a 10-m-long belt transect was arbitrarily placed along levees on the two long sides of the paddy field and the outlet side, respectively. Within each transect, three rows of rice plants were selected as a sampling area. Thus, we sampled exuviae from three transects in each paddy field (10 m × 3 rows × 3 sides; approximately 450 rice plants) using hand collection, and transported the samples to the laboratory for further identification, as described above. All Odonata species were identified to the species level, except for *Ischnura*. Although *I. asiatica* was the representative adult *Ischnura* species, we retained our identification to the genus level for this taxon because of difficulty in identifying the species solely from exuviae. We also measured the water depth in the same manner as described for 2017.

2.6. Statistical Analyses

We compared Odonata emergence rates between conventional and natural paddy fields using negative binomial generalized linear mixed models (GLMMs, link function = log) using the package lme4 [32] in RStudio ver. 1.2.5042 [33]. Water depth data were right-skewed and were square root-transformed prior to the analyses. We treated farming (conventional or natural) and mean water level (from the beginning of the survey until the peak emergence of each species) as fixed factors and region (i.e., block) as a random factor. We compared differences in odonate assemblages between conventional and natural paddy fields using the chi-square test. In all cases, we used $\alpha = 0.05$ to determine statistical significance.

3. Results

3.1. Preliminary Survey (2017)

In the 2017 survey, we identified six odonate taxa from the 16 paddy fields (Table 1). The most numerically dominant species was *S. frequens* (51.3%), followed by *S. infuscatum* (36.1%) and *S. darwinianum* (8.8%). Remaining species were represented by few individuals (<2.0% each).

Table 1. List of Odonata identified from exuviae in the study area in 2017. For each taxon, the sum of exuviae and their contribution (Cont.) and prevalence (Prev.) in all paddy fields are presented.

Taxon	Conventional Farming			Natural Farming		
	Exuviae (Number)	Cont. (%)	Prev. (%)	Exuviae (Number)	Cont. (%)	Prev. (%)
Lestes temporalis	2	1.5	12.5	-	-	-
Sympetrum darwinianum	5	3.7	12.5	16	15.7	37.5
Sympetrum infuscatum	71	52.2	37.5	15	14.7	37.5
Sympetrum frequens	58	42.6	75.0	64	62.7	100.0
Pantala flavescens	-	-	-	3	2.9	12.5
Orthetrum albistylum	-	-	-	4	3.9	25.0

When Odonata assemblages were compared between conventional and natural paddy fields, we found significant differences between the two farming types ($\chi^2 = 24.8$, $p < 0.001$) (Figure 2a). Four Odonata taxa were found in conventional paddy fields, with *S. infuscatum* (52.2%) and *S. frequens* (42.6%) representing the two numerically dominant taxa. In contrast, five Odonata taxa were identified from natural paddy fields, with *S. frequens* (62.7%), *S. darwinianum* (14.7%), and *S. infuscatum* (15.7%) representing the three numerically dominant taxa.

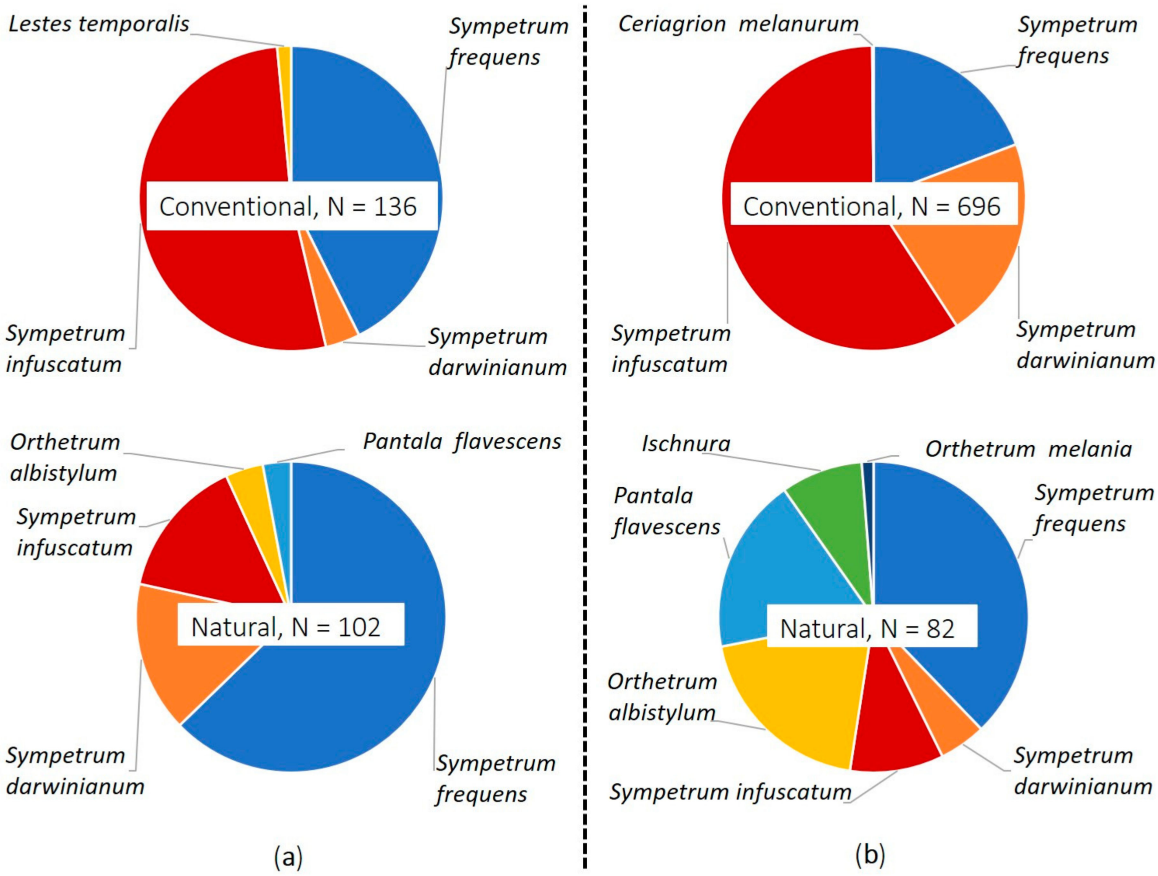

Figure 2. Odonata assemblage compositions in conventional and natural paddy fields in (**a**) 2017 and (**b**) 2019. The assemblage compositions differed between conventional and natural paddy fields in both 2017 (chi-square test, $\chi^2 = 24.8$, $p < 0.001$) and 2019 ($\chi^2 = 51.4$, $p < 0.001$).

Peak emergence dates for all Odonata differed between conventional and natural paddy fields. In conventional paddy fields, the total emergence rate (i.e., that of all species) was highest in late June. By contrast, in natural paddy fields, the total emergence rate was highest in early to mid-July (Figure 3). The emergence patterns of *S. frequens* and *S. infuscatum* generally reflected that of all Odonata in conventional paddy fields. In natural paddy fields, the emergence rate of *S. frequens* was highest in early July, followed by *S. infuscatum* in mid-July and *S. darwinianum* in late July.

GLMMs indicated that neither the farming practice nor mean water depth had a significant effect on the emergence of all Odonata, *S. frequens*, *S. infuscatum*, or *S. darwinianum* (Table 2).

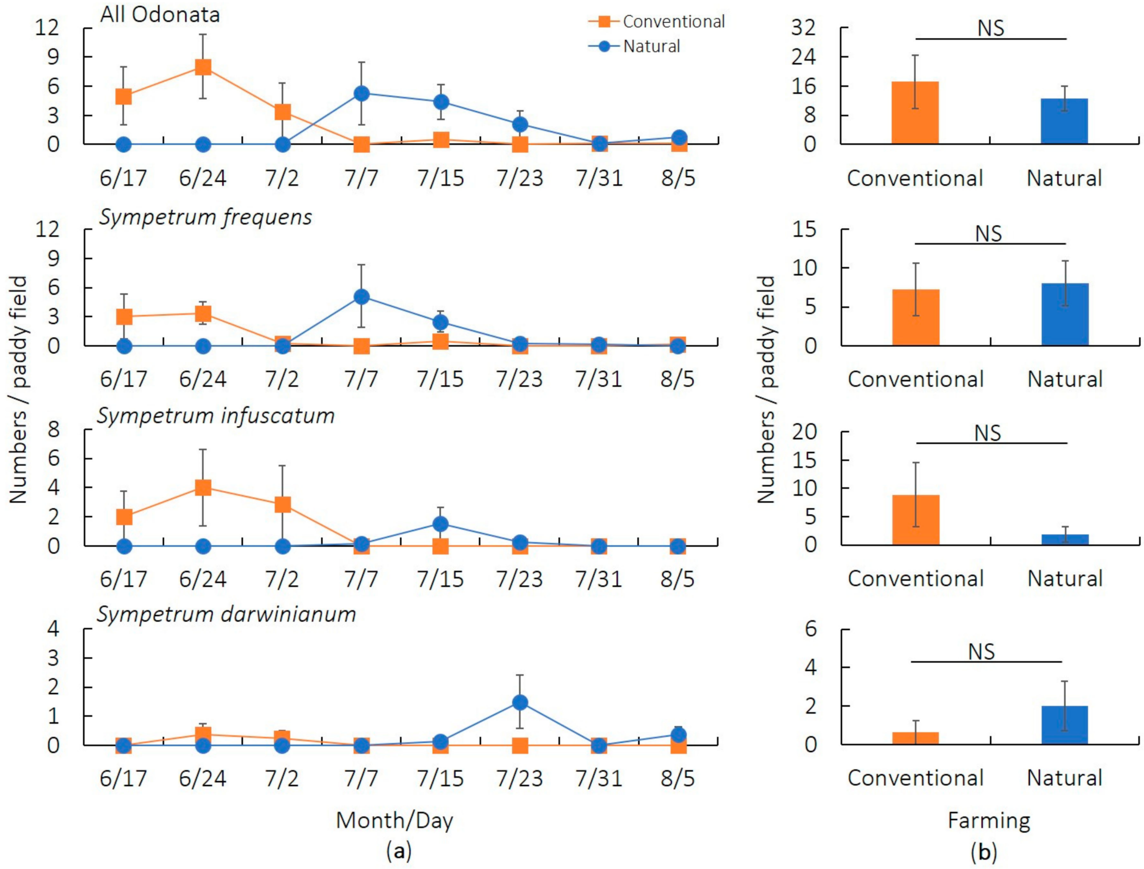

Figure 3. (**a**) The weekly mean (± SE) and (**b**) total mean (±SE) numbers of Odonata exuviae collected in conventional and natural paddy fields from June 17 to August 5, 2017; NS, not significant at α = 0.05.

Table 2. Summary statistics of generalized linear mixed models (GLMMs) that tested the effects of farming (conventional vs. natural) and mean water depth (until peak emergence) on the emergence rates of Odonata in 2017.

Response Variable	Covariate	Estimate	SE	z	*p*
Total Odonata	Intercept	2.586	0.439	5.888	<0.001
	Farming †	−0.852	0.835	−1.020	0.308
	Mean water depth	0.336	0.345	0.974	0.330
Sympetrum frequens	Intercept	2.008	0.477	4.214	<0.001
	Farming †	0.551	0.779	0.707	0.480
	Mean water depth	−0.233	0.328	−0.709	0.478
Sympetrum infuscatum	Intercept	1.520	0.913	1.665	0.096
	Farming †	−3.620	3.324	−1.089	0.276
	Mean water depth	1.005	1.230	0.817	0.414
Sympetrumdarwinianum	Intercept	1.520	0.913	1.665	0.096
	Farming †	−3.620	3.324	−1.089	0.276
	Mean water depth	1.005	1.230	0.817	0.414

† Conventional farming was treated as the reference.

3.2. Main Survey (2019)

We identified eight Odonata taxa across all 16 paddy fields in 2019 (Table 3). The most numerically dominant species in the study region was *S. infuscatum* (53.9%), followed by *S. frequens* (21.2%) and *S. darwinianum* (19.8%). Remaining species were represented by few individuals (<3.0% each).

As in 2017, odonate assemblages differed significantly between conventional and natural paddy fields (χ^2 = 51.4, $p < 0.001$) (Figure 2b). Four taxa were identified from conventional paddy fields, with *S. infuscatum* (59.1%), *S. darwinianum* (21.6%), and *S. frequens* (19.3%) representing the three numerically dominant taxa. By contrast, seven taxa were identified from natural paddy fields, with *S. frequens* (37.8%), *O. albistylum* (19.5%), *P. flavescens* (18.3%), *S. infuscatum* (9.8%), and *Ischnura* (8.5%) representing the five numerically dominant taxa.

Table 3. List of Odonata identified from exuviae in the study area in 2019. For each taxon, the sum of exuviae and their contribution (Cont.) and prevalence (Prev.) across all paddy fields are presented.

Taxon	**Conventional Farming**			**Natural Farming**		
	Exuviae (Number)	**Cont. (%)**	**Prev. (%)**	**Exuviae (Number)**	**Cont. (%)**	**Prev. (%)**
Ceriagrion melanurum	1	0.1	12.5	-	-	-
Ischnura	-	-	-	7	8.5	12.5
Sympetrum darwinianum	150	21.6	62.5	4	4.9	37.5
Sympetrum infuscatum	411	59.1	62.5	8	9.8	37.5
Sympetrum frequens	134	19.3	62.5	31	37.8	62.5
Pantala flavescens	-	-	-	15	18.3	50.0
Orthetrum albistylum	-	-	-	16	19.5	25.0
Orthetrum melania	-	-	-	1	1.2	12.5

As in 2017, we observed differences in the peak emergence timing between conventional and natural paddy fields (Figure 4). In conventional paddy fields, the emergence rates of all Odonata and the three *Sympetrum* species were highest in late June. In natural paddy fields, the overall emergence rate of all Odonata, represented mainly by *S. frequens*, was highest in early July; there was no measurable peak emergence of *S. infuscatum* or *S. darwinianum* due to low emergence numbers.

GLMMs indicated that the emergence rates of all Odonata, *S. infuscatum*, and *S. darwinianum* were lower in natural paddy fields compared with conventional paddy fields (Table 4). However, the emergence rate of *S. frequens* did not differ significantly between the two farming practices. Although the emergence rate of all Odonata was marginally positively associated with mean water depth ($p = 0.054$), this factor was not significant in explaining the emergence rates of any *Sympetrum* species. GLMM analyses could not be performed for *O. albistylum*, *P. flavescens*, or *Ischnura* because the emergence of these taxa was observed only in natural paddy fields.

Table 4. Summary statistics of generalized linear mixed models (GLMMs) that tested the effects of farming (conventional vs. natural) and mean water depth (until peak emergence) on the emergence rates of Odonata in 2019.

Response Variable	**Covariate**	**Estimate**	**SE**	**z**	***p***
Total Odonata	Intercept	3.089	0.725	4.263	<0.001
	Farming †	−2.850	0.837	−3.406	<0.001
	Mean water depth	0.839	0.436	1.925	0.054
Sympetrum frequens	Intercept	2.204	1.085	2.032	0.042
	Farming †	−2.012	1.269	−1.585	0.113
	Mean water depth	0.459	0.750	0.612	0.540
Sympetrum infuscatum	Intercept	2.438	1.153	2.115	0.034
	Farming †	−4.567	1.323	−3.452	<0.001
	Mean water depth	0.866	0.667	1.299	0.194
Sympetrumdarwinianum	Intercept	−0.556	1.272	−0.437	0.662
	Farming †	−3.935	0.626	−6.286	<0.001
	Mean water depth	0.687	0.429	1.603	0.109

† Conventional farming was treated as the reference.

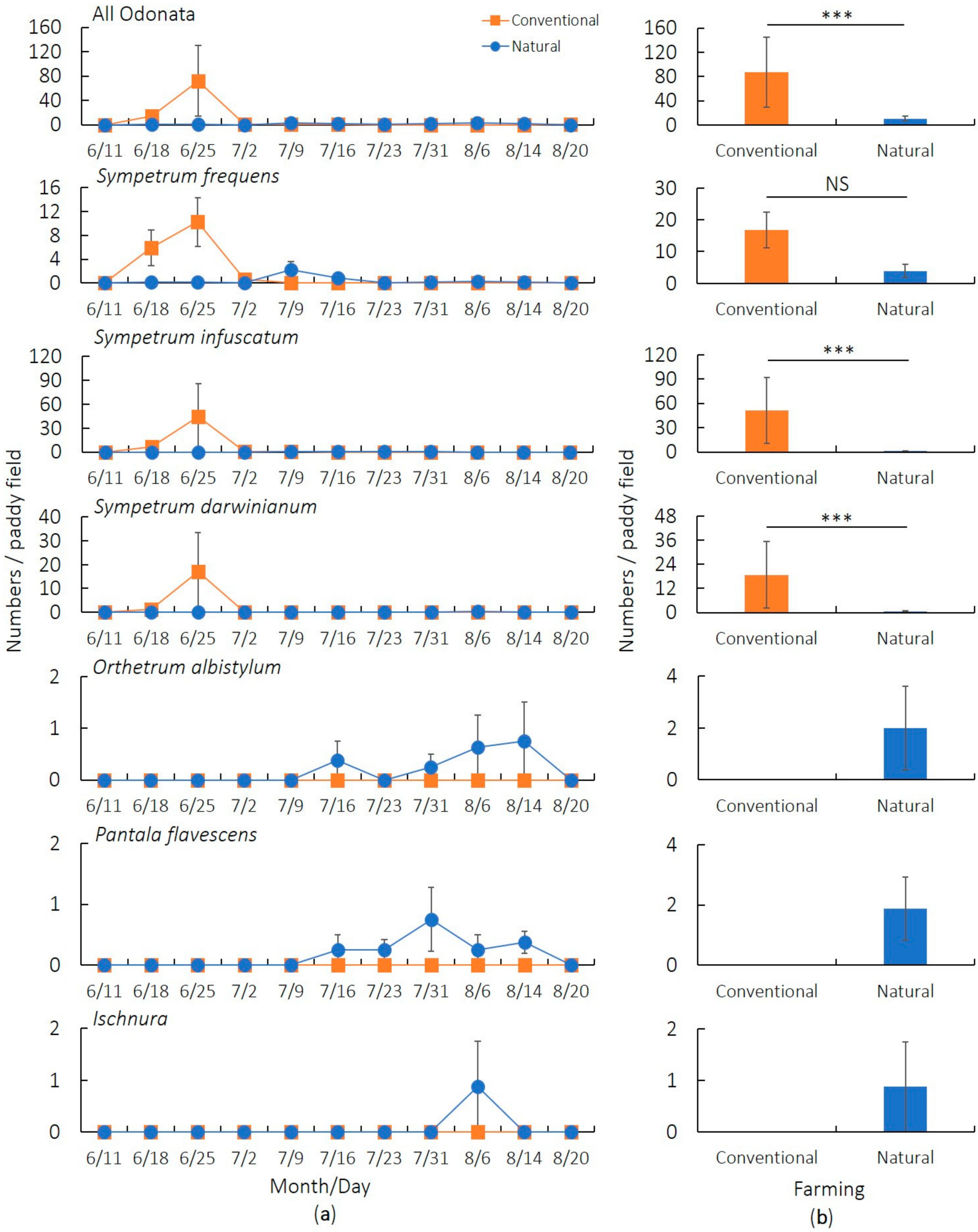

Figure 4. (**a**) The weekly mean (±SE) and (**b**) total mean (±SE) numbers of Odonata exuviae collected from conventional and natural paddy fields from June 11 to August 20 in 2019; ***, $p < 0.001$; NS, not significant at $\alpha = 0.05$.

4. Discussion

We found differences in Odonata assemblages between conventional and natural paddy fields, but both were numerically dominated by *Sympetrum* species. Conventional paddy fields were dominated numerically by *S. infuscatum* (2017 and 2019), *S. frequens*

(2017 and 2019), and *S. darwinianum* (2019), whereas natural paddy fields were primarily dominated by *S. frequens* in both sampling years. Although the abundance was low, more Odonata taxa emerged from natural paddy fields than from conventional paddy fields, especially in 2019 (Table 3). The three major non-*Sympetrum* taxa (*O. albistylum*, *P. flavescens*, and *Ischnura*) that emerged from natural paddy fields in 2019 are multigenerational taxa [28]. *Pantala flavescens* migrates from continental Asia, and the remaining two are resident taxa that exhibit larval overwintering [28]. This suggests that natural paddy fields serve as reproductive or larval overwintering habitats for some non-*Sympetrum* Odonata.

Generally, insecticides are applied to paddy fields in two different forms between spring and early summer under conventional methods: in nursery boxes in early to mid-May and aerial application prior to panicle initiation from late July to early August (Appendix A, Table A1). Some nursery box insecticides, such as neonicotinoids, have detrimental effects on the emergence of *Sympetrum* dragonflies [15,16]. Ferterra (chlorantraniliprole), which has been used in recent years in our study region, may differentially effect *Sympetrum* emergence depending on the species and water depth (Nakanishi et al., unpublished data). However, aerial insecticide application may have lethal effects across Odonata taxa. If larvae were present in conventional paddy fields during aerial application, it is unlikely that they would survive once water levels are reduced during midseason drainage (Appendix A, Figure A1), thereby increasing insecticide concentrations. Intermittency of water during midseason drainage may also have negative effects on the survivorship and emergence of Odonata in conventional paddy fields.

Contrary to our expectation, the overall emergence of Odonata did not differ between conventional and natural paddy fields in 2017. In 2019, the total number of emerging odonates was actually higher in conventional than in natural paddy fields. We also found that the timing of peak emergence differed between conventional and natural paddy fields; the emergence of odonates in conventional paddy fields peaked in late June and that in natural paddy fields peaked in early to mid-July. In our study, total Odonata emergence rates were reflective of the emergence patterns of the numerically dominant *Sympetrum* species. The differences in peak emergence timing between the farming methods is due mainly to differential timing of rice transplantation (and hence inundation) in the respective paddy fields. The life cycles of *Sympetrum* dragonflies are largely aligned with the rice farming calendar. Delayed rice transplantation and inundation under natural farming methods may not necessarily lead to enhanced emergence rates or abundance in these species.

Another potential explanation for the lower emergence of *Sympetrum* in natural paddy fields in 2019 may be enhanced predation. A study in Australia reported that although a greater diversity of macroinvertebrates was found in organic relative to conventional paddy fields in the early stages of cultivation, these communities tended to become more similar toward the middle and late cultivation stages due to increased predator abundance in organic paddy fields, including that of libellulid nymphs [17]. Given that we observed the emergence of non-*Sympetrum* taxa in natural, but not conventional, paddy fields, it is possible that greater larval competition for food resources or predator–prey interactions are occurring in natural paddy fields. Interspecific interactions in larval dragonflies have been reported among *Epitheca* spp. (Corduliidae), *Celithemis fasciata* (Libellulidae), and *Ladona deplanata* (Libellulidae) [34]. Interspecific interactions between *Sympetrum* and non-*Sympetrum* larvae may be of interest in future research.

A limitation of our study is its inability to disentangle various management factors. These include the intermittence or permanence of the water level and applications of pesticides and fertilizers. Studies have shown that midseason drainage has negative effects on aquatic insects, including Odonata [9,14]. Although we included the mean water level as an explanatory variable in the GLMM analyses, different water retention conditions in paddy fields may have different consequences for the timing and extent of drainage; some paddy fields dry up quickly and completely due to good drainage conditions, whereas others retain water for a long time before drying up or always retain water due to poor drainage. Such water conditions may also have different consequences for odonate species depending on their life stages. In addition, farmers apply various pesticides and fertilizers in conventional paddy fields. Nakanishi et al. [35] reported a significant negative relationship between the increased use of nursery box insecticides and the abundance of *Sympetrum* dragonflies (*S. frequens*). Nevertheless, studies evaluating the effects of pesticides are mostly limited to specific nursery box insecticides, such as neonicotinoids (reviewed in [16]). We have little knowledge of the effects of other pesticides, including insecticides applied from unmanned helicopters, herbicides, fungicides, and chemical fertilizers, on non-pest insects or animals. Owing to numerous confounding management effects, the effects of such management factors need to be evaluated experimentally.

The lack of consideration of adjacent or surrounding land-use types and the cultivation history is another limitation. Studies have reported that landscape attributes such as forest area and pond density around paddy fields contribute to Odonata diversity, depending on life-history stage [36–38]. Our study sites were generally located on plains (Figure 1). Nevertheless, the concentration of natural paddy fields might have affected the biodiversity of the surrounding paddy fields by serving as a source, known as the "spillover effect" [39]. Conversely, seepage of pesticides from surrounding paddy fields may also affect Odonata diversity regardless of the implementation of natural practice in a paddy field. Furthermore, the cultivation history (i.e., years of consecutive cultivation) with regard to natural farming may affect Odonata diversity. Such potential effects of the surrounding landscape features and cultivation history should be considered in future work.

5. Conclusions

We advocate that both conventional and natural paddy fields are important habitats for Odonata in our study area. Whereas conventional paddy fields serve as habitats for the three dominant *Sympetrum* species, which reach peak emergence in late June, natural paddy fields serve as habitats for *S. frequens*, which has a long emergence period. Although the overall emergence numbers were low, natural paddy fields also serve as habitats for various Libellulidae dragonflies and damselflies when conventional paddy fields are undergoing midseason drainage or insecticide application. Therefore, a mix of conventional and natural paddy fields may be desirable in supporting Odonata diversity in the paddy-dominated landscapes of rural Japan.

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Appendix A

Table A1. Major synthetic chemical pesticides (insecticides, fungicides, herbicides) and fertilizers used in conventional rice paddy fields in the Hakui–Houdatsushimizu area in 2019. Farmers are required to use a total of 15 or less active ingredients (indicated in square brackets) of pesticides and 5.6 kg/0.1 ha or less nitrogen fertilizer content. Data from JA Hakui.

Figure A1	Type of Agrochemical	Main Ingredient (Product Name in Japan) [Number of Active Ingredients]	Application Amount
Late March to late April	Seed disinfectant	Ipconazole–cupric hydroxide (Techlead C Flowable) [1] *Talaromyces flavus* SAY-Y-94-01 strain spore (Tafuburokku) [0]	200 times dilution
Early to mid-May	Nursery box insecticide	Chlorantraniliprole–probenazole (Dr. Orize Ferterra) [2] Chlorantraniliprole–tiazinil (Buiget Ferterra) [2]	50 g/nursery box
Late July to early August	Insecticide— fungicide	Ethofenprox–tricyclazole (Beam Eight Trebon Sol) [2] Echiprole (Kirappu Flowable) [1] Validamycin (Varidasin Air) [0]	800 mL/0.1 ha †
Early to mid-May	Paddy herbicide	Butachlor (Marchette 1 kg granules) [1] Imazosulfuron–oxazichromephone–pyraclonil (Sarabureddo KAI 1 kg granules) [3]	1.0 kg/0.1 ha 1.0 kg/0.1 ha
Late April to mid-July	Levee herbicide	Gluphosate potassium salt (Roundup Max Road) [1] Glufosinate (Basta) [1] Glyphosate isopropylamine salt (Sanfuron) [1]	300–500 mL/0.1 ha
Mid-March to early April Mid-April to mid-May Mid-June Mid-July	Soil conditioner Basal fertilizer Interim fertilizer Fertilizer for ear manuring	Magnesium–aluminum–silicic acid (BB Ta-no-megumi) Magnesium–aluminum–silicic acid (Super-keisan) Nitrogen–phosphorus–potassium–organic material (BB yuuki-iri Noto-koshi-ippatsu), Nitrogen–phosphorus–potassium– magnesium–silicic acid (BB keisan-power koshi-ippatsu-kun) Phosphorus–potassium– magnesium–silicic acid–boron (PK keisan) Nitrogen–phosphorus–potassium–magnesium (BB tsuihi 550-go)	40–60 kg/0.1 ha 30–50 kg/0.1 ha 40 kg/0.1 ha 13–20 kg/0.1 ha

† The amount applied through unmanned helicopter.

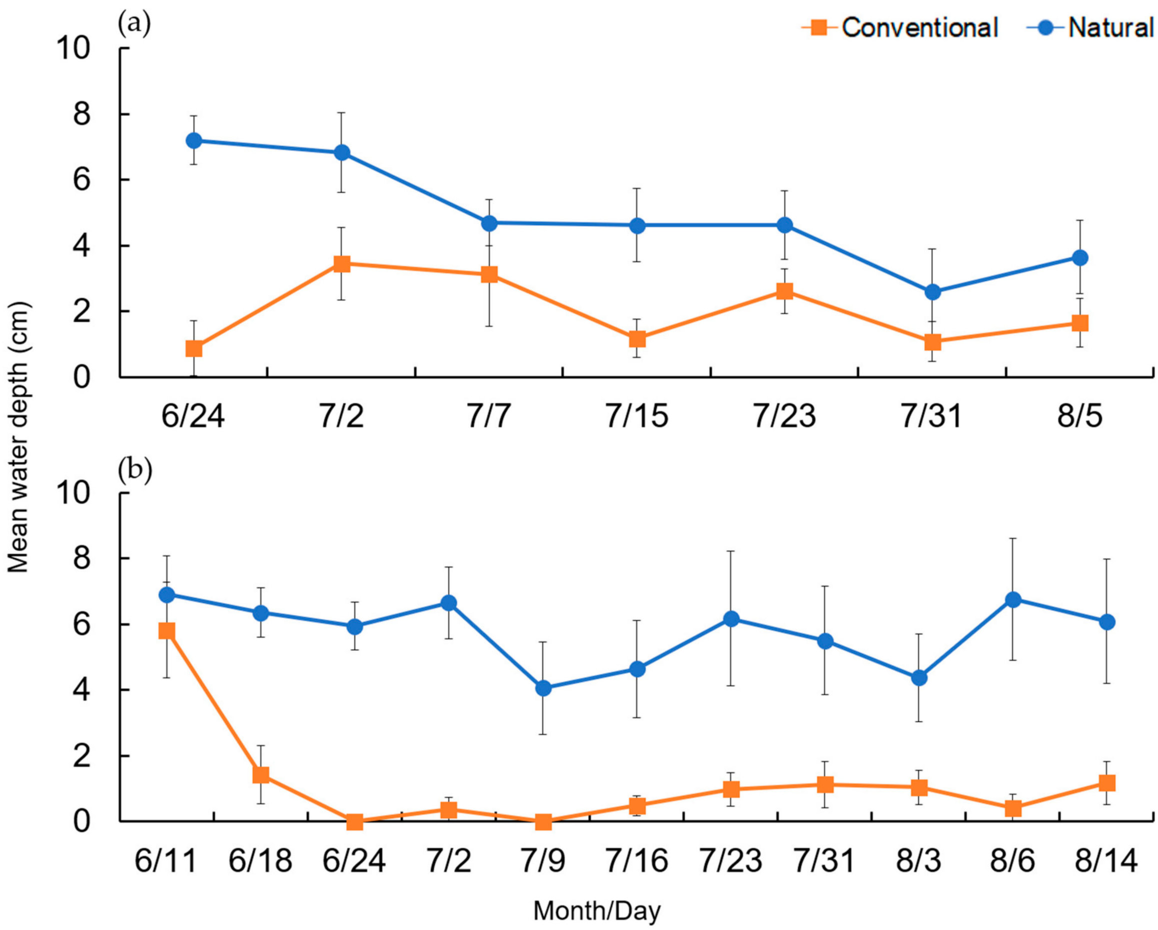

Figure A1. Mean water depth (± SE) in conventional and natural paddy fields in (**a**) 2017 and (**b**) 2019.

(**a**) (**b**)

Figure A2. Photos of (**a**) exuviae sampling and (**b**) *Sympetrum* exuviae.

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