Dissertation Outline

Effects of agrochemical–free rice farming on Odonata diversity

無農薬稲作への取り組みがトンボ類の多様性に与える影響

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In Japan, implementation of agrochemical-free farming is a sound means for restoration of degraded Satoyama. Through two-year field surveys, we investigated the emergence of Odonata in natural (agrochemical- and fertilizer-free cultivation) and conventional paddy fields. We found that assemblage compositions and peak emergence periods of Odonata differed between the two farms. Although the abundance was low, more odonate taxa emerged from natural than conventional paddy fields. In contrast, conventional paddy fields had equivalent or higher emergence rates of all Odonata and two *Sympetrum* relative to natural paddy fields. These results suggest that both paddy field types are important habitats for Odonata.

Ground bamboo (GB) application has been shown to have high potential in increasing rice yield. Nevertheless, its effects on paddy field biodiversity are unknown. Through two-year field experiments with exuviae and environmental DNA analyses, we evaluated the effects of GB application on Odonata diversity. The taxonomic richness and total abundance of Odonata did not differ among treatments. However, GB application had mix effects on odonate emergence depending on taxa and applied GB volume. When GB are to be applied in working paddy fields, a suitable application volume should be determined to balance rice production and Odonata conservation.

Chapter 1: General Introduction

Rice is among the most important foods in the world. Over 90% of rice produced in Asia between 1994 and 2019 have fed millions of people in the world (FAOSTAT. 2020). To meet the food needs of the growing demand, vast areas of natural wetlands (e.g. floodplains) have been converted to rice paddy fields worldwide. For example in Japan, land-use change led to a 61% decline of natural floodplain wetlands over the past century (Geographical Survey Institute. 2000). Thus, biodiversity conservation in agricultural landscapes is a challenging theme in the field of Biodiversity Conservation.

In the last decades, two land management strategies have been proposed to restore degraded agricultural landscapes (Grass et al. 2019, reviewed in Loconto et al. 2020). The land sparing approach promotes crop production and conservation on separate land, and thereby promote intensive agriculture and biodiversity conservation in an agricultural landscape. In contrast, the land sharing approach promotes crop production and biodiversity conservation on the same agriculture land by implementing traditional or low-input (e.g. organic) farming practices. Deciding which land management strategy would be appropriate is context dependent. For example, land sparing would be appropriate in conserving species that are incompatible with agricultural activities, while land sharing would be suitable for conservation of endangered farmland species (Grass et al. 2019).

Increased attention has been given to Satoyama, traditional socio-ecological production landscapes in Japan, as a model for land sharing. Satoyama landscapes comprise paddy fields, cropland, farm ponds, streams and ditches, secondary forests, and grasslands around human settlements in rural areas (Usio and Miyashita 2014). Although paddy fields were primarily created for rice production purposes, these anthropogenic wetlands also serve as refuge habitats for aquatic and semi-aquatic wildlife, such as plants, water birds, fish, amphibians, and insects (reviewed in Natuhara, 2013).

However, in recent decades, Satoyama landscapes are facing two counteractive drivers of habitat degradation; modernization and abandonment of farming (Natuhara 2013). Modernization of farming has caused habitat loss and degradation through farmland consolidation and overuses of agrochemicals. In contrast, depopulation and aging in rural communities promoted abandonment of farming and farmland management, that in turn, led to habitat degradation through promotion of succession and loss of moderately disturbed habitats. By reducing native species and their habitats, these counteractive drivers also facilitated invasion and expansion of nonnative invasive species. For example, range expansion of moso bamboos (*Phyllostachys pubescens*) in rural Japan is among major threats for loss of biodiversity and ecosystem services in forests and farmland (reviewed in Xu et al. 2020). Developing strategies to effectively use bamboo biomass resources is warranted.

Traditionally, Satoyama served as a model for resource circulation (Takeuchi 2010). For example, firewood and charcoal from the forest used to provide fuel resources for cooking and heating, while agricultural residues were used to provide food for livestock (Takeuchi 2010). Through promotion of resource circulation, developing strategies to effectively use bamboo biomass resources in low-input rice farming, is expected to promote management of non-native bamboos, mitigation of environmental burden by reducing the use of agrochemicals, and conservation of biodiversity.

Chapter 2: Do agrochemical–free paddy fields serve as refuge habitats for Odonata?

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*

Chapter 3: Application of bamboo biomass resources in agrochemical–free rice farming: effects on Odonata diversity

In recent years, there are growing interests in using bamboo biomass in agriculture. Bamboo biomass has been reported to effectively suppress weed biomass and increase rice yield in agrochemical-free rice farming. In this study, we evaluated the effects of ground bamboo application on paddy field biodiversity using Odonata as an indicator. We conducted field experiments over two summers using 16 experimental paddies comprising five treatments with 3–4 replicates each: control (no weeding or ground bamboo application), manual weeding (weeds were manually removed once in summer, no ground bamboo application), low-volume ground bamboo application (0.5 t/0.1 ha), medium-volume ground bamboo application (1.0 t/0.1 ha), and high-volume (2.0 t/0.1 ha) ground bamboo application. Rice plants (*Oryza sativa*) were transplanted to the 16 experimental paddy fields. During the cultivation period, we did not use pesticides or fertilizers (either inorganic or organic) other than ground bamboos in the ground bamboo treatments. In the summers of 2018 and 2019, we carried out weekly exuviae sampling and environmental DNA (eDNA) analyses. At given times, greater numbers of Odonate taxa were identified by eDNA relative to exuviae analyses. Neither eDNA nor exuviae analyses showed significant difference in Odonata richness among treatments. On the basis of exuviae analyses, total Odonata abundance did not differ among treatments. However, the emergence rates of three Libellulidae dragonflies (*Sympetrum frequens*, *Crocothemis mariannae*, and *Pantala flavescens*) were low or zero in the high-volume ground bamboo treatment. In contrast, the emergence rates of *Anax parthenope* (Aeshnidae) were highest in the high-volume ground bamboo application treatment. These results suggest that application of ground bamboo have mixed effects on Odonata emergence depending on odonate taxa and volume of ground bamboos applied. A suitable application volume of ground bamboos should be determined to balance rice production and Odonata conservation.

Keywords: Dragonfly; Damselfly; *Phyllostachys pubescens*; Mulching; Resource circulation farming; Organic farming

Chapter 4: Summary

Throughout this study, key findings are as below:

- The Odonata assemblages differed between conventional and natural paddy fields, but both were numerically dominated by *Sympetrum* species. Generally, conventional paddy fields were dominated numerically by *S. infuscatum*, whereas natural paddy fields were primarily dominated by *S. frequens* (Chapter 2).
- 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 (Chapter 2).

- 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 (Chapter 2).
- Although the abundance was low, more Odonata taxa emerged from natural paddy fields than from conventional paddy fields, especially in 2019 (Chapter 2).
- Application of ground bamboos led to anti-oxidizing conditions in experimental paddy fields in the early cultivation periods (Chapter 3).
- Aquatic weeds were not effectively suppressed in the ground bamboo application treatments in experimental paddy fields regardless of the volumes of ground bamboos applied (Chapter 3).
- Exuviae and e DNA analyses in 2018 and 2019 revealed that Odonata richness and abundance (total Odonata and dominant Odonata taxa) did not differ among treatments. However, the emergence rates of three Libellulidae dragonflies (*S. frequens*, *Crocothemis mariannae*, and *Pantala flavescens*) were low or zero in the high-volume ground bamboo treatment in two years (Chapter 3).
- When the results from exuviae and eDNA analyses were compared, 7 out of 10 taxa in 2018 and 5 out of 10 taxa in 2019 were commonly found in both exuviae and eDNA analyses (Chapter 3). At given times, more Odonata taxa were identified by eDNA relative to exuviae analyses.

Prospects

Based on these findings and limitations, I would propose several topics for future studies including:

- Mechanisms of positive or negative responses to specific agrochemicals or fertilizers by considering different susceptibility to agrochemicals or water quality as well as predator-prey interactions among Odonata and other animals in food webs.
- Effects of local factors (intermittence or permanence of the water level, applications of pesticides and fertilizers, year of natural farming) and landscape factors (surrounding land uses, pond density) on Odonata diversity at field scale.
- Developing priority restoration sites for wildlife-friendly rice farming with reference to land-use or landscape factors.
- Larval sampling and adult observation may be ideally conducted together with exuviae sampling in future studies for clarifying true positives and contributions of odonate diversity at different growing stages.

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 (Chapter 2).

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

Table 2. 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 (Chapter 2).

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

Table 3. 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 (Chapter 2).

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
Sympetrum darwinianum	Intercept	-8.024	6.250	-1.284	0.199
	Farming†	-1.970	1.630	-1.208	0.227
	Mean water depth	3.715	2.344	1.585	0.113

† Conventional farming was treated as the reference

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 (Chapter 2).

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
Sympetrum darwinianum	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

Table 5. List of Odonata identified from exuviae in the paddy field experiment in 2018 and 2019. For each taxon, the sum of exuviae, the percentage contributions (Cont.), and the number of reads detected by environmental DNA (eDNA) in all paddy fields are presented (Chapter 3).

Taxon	**2018**			**2019**		
	Exuviae (Number)	**Cont. (%)**	**eDNA (Number of reads)**	**Exuviae (Number)**	**Cont. (%)**	**eDNA (Number of reads)**
Aeshnidae						
Aeshna mixta	3	0.3	–	–	–	–
Anax parthenope	39	3.5	6,284	28	5.6	10,840
Calopterygidae						
Mnais pruinosa	–	–	–	–	–	758
Coenagrionidae						
Ceriagrion melanurum	1	0.1	310	–	–	38
Ischnura *	796	71	1,564,925	306	61	60
Libellulidae						
Crocothemis mariannae	55	4.9	48,968	17	3.4	252,077
Lyriothemis pachygastra	–	–	1,198	–	–	104
Orthetrum albistylum	137	12	121,654	37	7.4	113,970
Orthetrum japonicum	–	–	1,466	–	–	687
Orthetrum melania	–	–	22	–	–	126
Pantala flavescens	19	1.7	14,960	17	3.4	–
Sympetrum eroticum	1	0.1	–	–	–	–
Sympetrum frequens	71	6.3	1,846	95	19	326
Platycnemididae						
Copera annulata	2	0.2	–	–	–	–

* Taxon identified to the genus level only by exuviae identification, identity of eDNA analyses of this taxon was less than 99%.

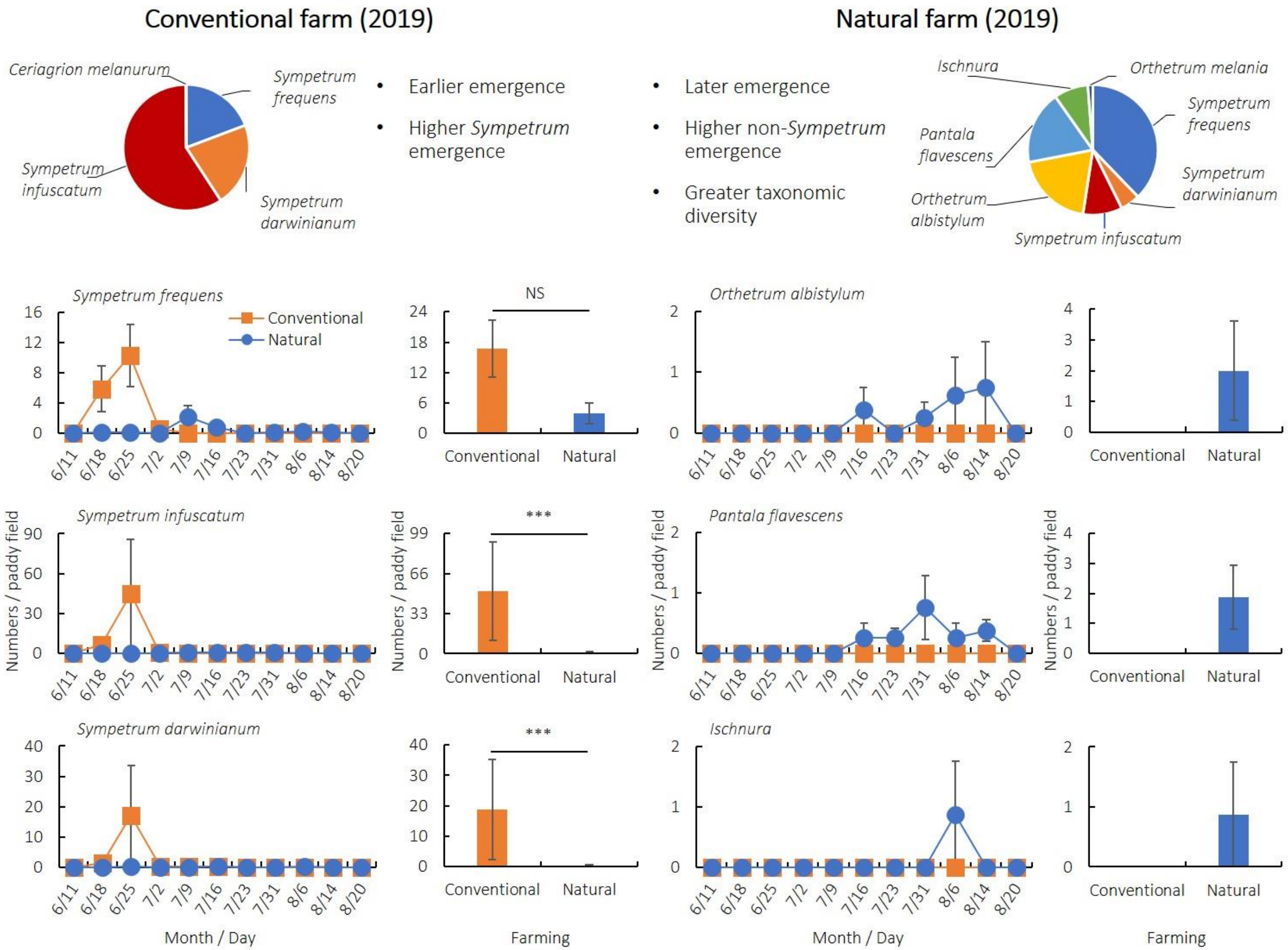

Figure 1. Odonata assemblage compositions in conventional and natural paddy fields in 2019 (pie charts) (chi-square test, $\chi 2 = 51.4$, $P < 0.001$). The weekly mean (± SE) (line charts) and total mean (± SE) (bar charts) numbers of Odonata exuviae collected in conventional and natural paddy fields from June 11 to August 20 in 2019; ***, $P < 0.001$; NS, not significant at $\alpha = 0.05$.

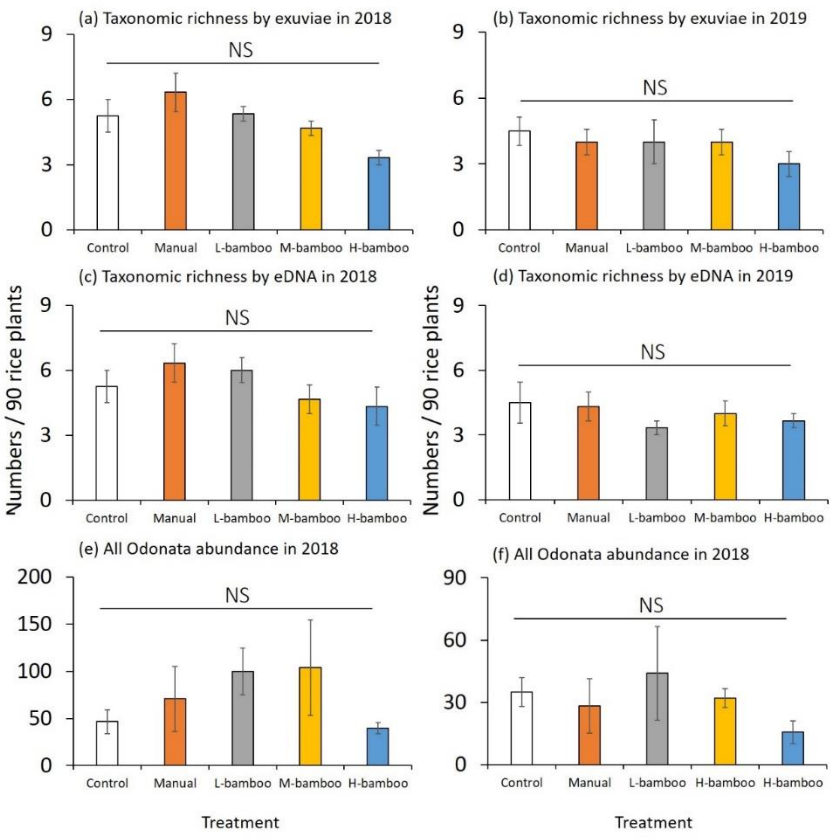

Figure 2. Odonata taxonomic richness by exuviae identification (a,b) and environmental DNA (eDNA) (c,d) and all Odonata abundance (e,f) in the paddy field experiments in 2018 and 2019. Data are the mean ± SE. NS, not significant. L-bamboo, low-volume ground bamboo application treatment; M-bamboo, medium-volume ground bamboo application treatment; H-bamboo, high-volume ground bamboo application treatment (Chapter 3).

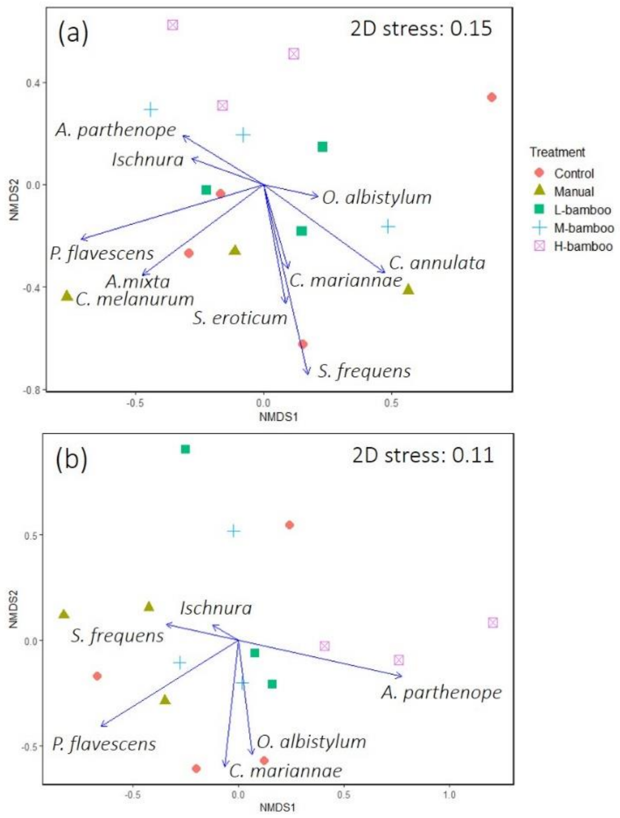

Figure 3. Nonmetric multidimentional scaling (NMDS) ordinations based on the abundance of Odonata in the paddy field experiments in (a) 2018 and (b) 2019. 2D stress indicates the model fit. L-bamboo, low-volume ground bamboo application treatment; M-bamboo, medium-volume ground bamboo application treatment; H-bamboo, high-volume ground bamboo application treatment (Chapter 3).