A Comparison of Aquatic Invertebrate Diversity between Paddy Fields under Traditional and Modern Management in Western Japan

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Abstract
We compared aquatic invertebrate diversity in paddy fields under traditional and modern management practices. We also examined the general factors that may be related to diversity within each paddy field. The Kitadan area was farmed with traditional management practices, with much longer periods of irrigations compared with the Kahoku area, which was farmed with modern agricultural practices using intermittent irrigation. We measured physicochemical factors and collected aquatic invertebrates from the two areas. Collected animal specimens were taxonomically identified and classified based by desiccation tolerance ability. At Kitadan, the total biodiversity parameters increased over time, while, biodiversity fluctuated temporally at Kahoku. The mean Shannon-Wiener diversity index was higher at Kitadan than at Kahoku. However, the mean taxon richness was not significantly different between locations. Both paddy fields were dominated by highly desiccation-tolerant invertebrates at the beginning of the rice cultivation period. Over time, the ratio of desiccation-sensitive invertebrates increased more at Kitadan than at Kahoku. Our canonical correspondence analysis showed that the time since rice transplanting, water permanence, pH, water depth, and chlorophyll a were significant factors affecting faunal assemblage composition. Our results indicate that water management practices have important roles in the aquatic biodiversity of paddy-field ecosystems.

Keywords
biodiversity, water permanence, satoyama, desiccation tolerance

1. Introduction
Paddy fields are important agricultural ecosystems that provide food for humans and promote the diversity of plants and animals (Fernando, 1993). Japanese paddy fields occupy > 24,580 km² of landscape (Statistics of 2014, Japanese Ministry of Agriculture, Forestry and Fisheries), an area tenfold greater than the surface cover of natural lakes and ponds in Japan (2300 km², considering only water bodies ≥ 1 km² in area; Geospatial Information Authority of Japan). The high biodiversity of this huge
artificial ecosystem is under threat from ongoing changes in Japanese agricultural practices. Flat lands with adequate water supplies for rice cultivation are scarce in Japan. Traditional Japanese farmers have used the bottoms of small valleys as paddies, which are referred to as “yatsuda” or “yachida” in the Japanese language. Typical “yatsuda” are small terraced paddies irrigated by springs that are supplied by the watersheds in the surrounding forests. “Satoyama” is a Japanese term that encompasses the whole traditional agricultural landscape of Japan. Satoyama comprises a mosaic of diverse vegetation, including secondary oak forests, grassland, bamboo groves, and paddy fields. In the past, the components of this diverse vegetation were closely coupled and were essential for maintaining the livelihoods of the human population living in the landscape (Tabata, 1997).

Traditional “yatsuda” rarely suffered drought during the rice cultivation period, and irrigation was constant. In modernized paddy fields, intermittent irrigation is encouraged to provide oxygen to the roots of rice plant and to enable the use of heavy machines in the fields (JSIDRE, 1992). In recent years, “yatsuda” have been abandoned in favor of more modernized agriculture management. To reduce manual labor, conventional agricultural practices in Japan have changed markedly in recent years; intermittent irrigation in summer, direct irrigation systems with pipes and taps, and the application of agrochemicals to nursery boxes are all components of this shift in arable procedures (Fujioka & Lane, 1997; Ministry of Agriculture, Forestry and Fisheries of Japan, 2005; Simpson & Roger, 1995).

The effects of these changes in agricultural practices on the terrestrial and aquatic fauna of paddy fields have been investigated over the last 20 years. Intermittent irrigation and modern water supply engineering threaten many aquatic organisms by reducing their access to the paddy fields (Lane & Fujioka, 1998; Washitani, 2007). Declines in fishes and invertebrates in modern paddy fields have been reported; these declines indirectly reduce the number of foraging birds (Narusue & Uchida, 1993).

Unfortunately, the fauna of traditional Japanese paddy fields was not well documented. Nevertheless, it is important to quantify specific impacts of water management changes in the paddy field ecosystem. The differences between modern and traditional agro-ecosystem may be attributable not to single variables but rather to a synthesis of multiple closely related factors. Among these is the “yatsuda” requirement for forest watershed land in close proximity, which restricts the size of individual paddy fields because the water supply must be local. Although it is important to describe the faunistic contrasts between the “yatsuda” and modern paddy farms, it is unlikely that the differences will be attributable to single, simple factors.

In this study, we compared aquatic invertebrate diversity in paddy fields under traditional management with that under modern management. We repeatedly sampled the invertebrates through the cultivation period to determine the faunal characteristics of the two systems. We also aimed to identify general patterns in the environmental variables that influenced biodiversity by studying the relationships between potential explanatory factors and the faunal attributes within and between the two agricultural systems.
2. Method

2.1 Study Sites

We selected two Japanese study areas: (i) the Kahoku flat lands (36°39'N, 136°40'E) (Figure 1A) in Kahoku City, and (ii) the small Kitadan valley (36°32'N, 136°42'E) (Figure 1B) located 15 km south of Kahoku in Kanazawa City; both areas were located in Ishikawa Prefecture. Paddy fields in Kahoku represented modernized management procedures (Figure 1A), and those in Kitadan operated under the traditional “yatsuda” system (Figure 1B). Paddy fields in Kahoku were intermittently irrigated in July and August from agricultural supply ditches using a pump and pipe system. The terraced paddy fields at Kitadan were located inside the “satoyama” zone of the Kanazawa University campus, where they were continually irrigated by a stream flowing from the upper to lower rice fields until harvesting took place.

In addition to the contrasting irrigation systems, the surrounding environments and spatial scales of the paddy field sites differed between areas. The Kahoku area was on land drained in 1985; at the time of our study, it supported grasslands, croplands, and paddy fields occupying a total area of 1100 ha. Each paddy field occupied ca. 1.5 ha; the total area under rice was > 86 ha (Figure 1A). Application frequencies and quantities of insecticide, fungicide, and nursery chemicals varied among the farmers, who shared a uniform irrigation system comprising pumped water supplied by pipes and controlled by taps. Farmers plowed in fertilizer during spring before rice transplanting, and practiced intermittent irrigation during the pre-harvest period (July-August) to stimulate rice growth. The fields were drained during harvesting and remained so to the next cultivation period (i.e., drained from September to April, Figure 2A). We chose three blocks 1 km apart along a 2 km east-west transect (Figure 1A) and collected samples from 15 sites on each sampling occasion, giving a total of 90 samples during the collection period.

Kitadan is a small valley surrounded by secondary deciduous forest. The area comprises ca. 20 terraced paddy fields, each 20-30 m² in area. The total area under rice occupied < 1 ha. Several artificial shallow ponds were in the vicinity (Figure 1B). Fields were managed by volunteers enrolled in an educational program operated by Kanazawa University. In autumn, after rice harvesting, cow manure was applied and plowed into the ground with remaining rice roots and stubble; the land was left dry until January (Figure 2B). The fields were irrigated from January until August from a stream flowing into the upper fields and down the slope to the lower fields. The water supply was insufficient to flood all the paddies during summer. Nevertheless, the soil was wet in August, even in the absence of standing water. No insecticides, herbicides, or nursery chemicals were used in these fields. We sampled 15 sites among 20 paddy fields on each sampling occasion, to give a total of 90 samples over the whole collection period.

2.2 Physicochemical Measurements

Our first measurements were made 1 week after rice nursery planting in the two areas. Aquatic invertebrates and water samples were collected six times at 2 week intervals from May to the early part of August in 2008. We recorded water depths and rice plant heights within each paddy field unit where we had previously sampled water and invertebrates.
As intermittent irrigation was practiced at 2 week intervals at Kahoku, we determined water permanence fortnightly. Water permanence was classified as “1” or “0”. When water was present in a field on two consecutive sampling occasions, the field was assigned water permanence category “1”, whereas when a field was dry over a 2 week period, it was assigned water permanence category “0”. All of the initial category assignments at Kahoku were “0” because flooding had taken place < 2 weeks previously. We observed no temporal variation in water permanence at Kitadan. The sites never dried out during our sampling period through early August.

We collected 50 mL samples of paddy field water in bottles, which we transferred to an insulated box and transported to the laboratory for measurements of chlorophyll a, turbidity, and pH. Chlorophyll a and turbidity were measured with a fluorometer (Aquafluor, Turner Designs, Sunnyvale, CA, USA), and pH was measured using pH indicator strips (Whatman CF, pH 4.5-10, GE Healthcare Life Sciences, Pittsburgh, PA, USA).

2.3 Aquatic Invertebrate Sampling

Aquatic invertebrates were collected using the dip scoop procedure. Part of dipper (500 mL volume, 15 cm diameter, 3 cm depth) was submerged in the water, causing an inflow. The dipper was immediately lifted above the surface before any outflow occurred. In this way, we captured invertebrates regardless of their motility. Approximately 10 dips (5 L total volume) were collected from the peripheries of the paddy fields at 2 m intervals along the shorelines. Collected water was filtered through a mesh (0.20 ± 0.01 mm) to concentrate the contents. The filtered invertebrate samples were transferred to 200 ml plastic containers (77 mm diameter, 57 mm height) and kept cool in an insulated box during transport to the laboratory. There, invertebrates were sorted, preserved in 1% formalin, and held in a refrigerator prior to identification. Specimens were identified using taxonomic keys and classified as desiccation sensitive or desiccation resistant depending on whether they had desiccation-tolerant life stages (William, 2006).

Biomass (mg dry weight) was calculated after sorting invertebrates by taxonomic identity and body size. We measured the dry weights of individual large animals using an analytical balance. Smaller invertebrates, such as zooplankton, were pooled into groups (> 20 individuals/group) for measurements. Specimens were stored in a desiccator at room temperature for > 48 h to constant weight before measurements were taken.

2.4 Data Analyses

Taxon richness, the Shannon-Wiener diversity index, and total invertebrate biomass were calculated for each sampling occasion to compile time series fluctuations in biodiversity for each type of paddy field. We used a cluster analysis of presence/absence data based on the Sørensen similarity index; the unweighted pair group procedure using the arithmetic mean (UPGMA) method was employed to examine similarities in animal taxonomic composition among paddy-field areas. The two biodiversity indices were calculated using PAST statistical software (Hammer et al., 2001). Because the data were not normally distributed, comparisons of diversity parameters between locations were conducted using
the non-parametric Mann-Whitney U-test (JMP, 2003). Indicator species and species compositions characterizing each of the paddy fields were determined using the IndVal method (Dufrene & Legendre, 1997). IndVal indicators were calculated using the labdsv package in R statistical software. Only species with indicator values > 0.25 (25%) and p-values < 0.05 are presented in our data. Canonical Correspondence Analysis (CCA) was used to relate the abundance of the animal taxonomic groups to environmental factors, i.e., chlorophyll a, turbidity, pH and water depth. We used CANOCO version 4.5 software (Microcomputer Power, Ithaca, NY, USA) for this analysis. The procedure allowed us to identify significant environmental factors determining the faunal assemblages in the paddy fields. Invertebrates densities were log transformed for the analysis. We determined the significance of relationships between faunal density and environmental variables using Monte Carlo permutation tests (n = 500).

3. Result

3.1 Community Composition

The aquatic invertebrate taxa we encountered are listed in Table 1. In total, 18 families, 11 orders, and seven classes were encountered at Kahoku; at Kitadan, we found members of 20 families, 10 orders and six classes. Branchiopod crustaceans such as *Moina* spp. (Moinidae, Cladocera) dominated density counts (76 indiv./L) and frequencies (64 positive samples of a total of 90) at Kahoku (Table 1). In contrast, the ostracod *Ilyocypris* spp. (Ilyocyorididae, Podocopida) dominated the density counts (18 indiv./L), and *Cyclopidae* sp. (Copepoda) dominated the frequency estimates (67 of 90) at Kitadan (Table 1). Similar numbers of insects were sampled at Kahoku and Kitadan (1.12/L and 1.17/L respectively). *Micronecta* sp. (Corixidae, Hemiptera) and *Anisops* sp. (Notonectidae, Hemiptera) dominated densities at Kahoku (2.8 indiv./L, Table 1), whereas *Rheotanytarsus* sp. (Chironomidae, Diptera) dominated frequency estimates (36 of 90, Table 1). The larval stage of *Cloeon dipterum* (Baetidae, Ephemeroptera) dominated densities and frequencies at Kitadan (3 indiv./L, 42 of 90, Table 1).

The cluster analysis grouped the samples (other than sample 20 from Kitadan) into two clusters associated with the two sites (Figure 3). Thus, we detected area-specific assemblages, which also emerged in the IndVal analysis (Table 2). Table 2 lists the differences in indicator species composition between Kahoku and Kitadan. Species assemblages characterizing Kahoku paddy fields comprised three taxa from the order Cladocera (Branchiopoda), one from the Diptera (Insecta), and one from the Architaenioglossa (Gastropoda). Species assemblages characterizing Kitadan paddy fields comprised two taxa of Ephemeroptera (Insecta) and one taxon of the Podocopida (Ostracoda). The taxa with the highest indicator values in Kahoku and Kitadan were *Moina* spp. (0.709, Table 2) and *Cloeon dipterum* (0.452, Table 2), respectively.

Biodiversity measured as taxon richness and the Shannon-Wiener index $H'$ varied within areas and between sampling times (Figure 4). Taxon richness in Kahoku fluctuated between sampling months,
whereas values at Kitadan increased continually, reaching a peak in late June (Figure 4). Mean $H'$ values also fluctuated variably at Kahoku, reaching a peak in June before decreasing again in July (Figure 4). In contrast, $H'$ values at Kitadan increased continually between May and June, and plateaued after July (Figure 4). $H'$ was always higher at Kitadan than at Kahoku ($n = 90$, Mann-Whitney U-test, $p = 0.0002$). Taxon richness, however, was not significantly different between sites ($n = 90$, Mann-Whitney U-test, $p = 0.901$). Total invertebrate biomass values were in the range 245-1435 mg at Kahoku and 333-1401 mg at Kitadan (Figure 4). The biomass time series differed between the two locations. The biomass at Kahoku was highest on the first sampling occasion and decreased thereafter through August. In contrast, values at Kitadan tended to increase until July, before decreasing in August.

3.2 Desiccation Tolerance

The proportions of animals that were desiccation tolerant during the May-August period are shown in Figure 5. Desiccation-tolerant taxa dominated the fauna during the early part of the cultivation season, whereas desiccation-sensitive taxa increased over time at both sites (Figure 5). The proportion of desiccation-sensitive members increased more quickly at Kitadan than at Kahoku (Figure 5). Desiccation-sensitive members were significantly more abundant (Mann-Whitney U-test, $p = 0.0001$) at Kitadan (30%) than at Kahoku (1.3%).

3.3 Relationships between Environmental Variables and Animal Assemblages

We applied a CCA to the whole dataset and found that “area” (Kahoku vs. Kitadan) was the most powerful explanatory variable ($F = 22.34$, $p = 0.002$, Table 3). To determine whether any of the general environmental parameters measured might explain the variation in animal assemblages, we applied a partial CCA using the variable “area” as a covariate, and showed that the animal assemblages were significantly related to the measured environmental factors (Table 3, Figure 6). The number of days after transplanting ($F = 9.54$, $p = 0.002$, Table 3), water permanence ($F = 3.15$, $p = 0.002$, Table 3), pH ($F = 2.96$, $p = 0.004$, Table 3), water depth ($F = 2.22$, $p = 0.010$, Table 3), and chlorophyll $a$ concentrations ($F = 1.89$, $p = 0.04$, Table 3) affected animal assemblage composition in both areas.

### Table 1. Mean Density and Frequency of Animal Assemblages in Kahoku and Kitadan

| Phylum | Desiccation tolerance | Kahoku $(N = 90)$ | Kitadan $(N = 90)$ |
|--------|-----------------------|------------------|------------------|
|        | Conventional Taxa     | Mean /litre freq | Mean /litre freq |
| Class  | Order | Family |                      |                  |
|--------|------------|---------|-----------------------|------------------|
| Arthropoda |                |         |                       |                  |
| Malacostraca | Amphipoda | Gammaridae | Gammaridea sp. | 1 | 0.2 | 2 | 0.4 | 7 |
| Maxillopoda | Copepoda | Cyclopidae | Cyclopidae sp. | 1 | 2.6 | 54 | 2.6 | 67 |
| Ostracoda | Podocopida | Cyprididae | Cyprinotinae spp. | 1 | 2.5 | 37 | 4.3 | 53 |

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| Kingdom     | Class                                  | Order                        | Family            | Genus                        | Species | Length | Width | Height | Count |
|-------------|----------------------------------------|------------------------------|-------------------|------------------------------|---------|--------|-------|--------|-------|
| Cnidaria    | Hydrozoa                               |                              |                   |                              |         |        |       |        |       |
|             | Anthomedusae                           |                              |                   |                              |         |        |       |        |       |
| Molusca     | Gastropoda                             |                              |                   |                              |         |        |       |        |       |

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### Table 2. Indicator Species Analysis (IndVal) of Invertebrates Collected in Kahoku and Kitadan

| Site   | Taxon             | IndVal | P value | Frequency |
|--------|-------------------|--------|---------|-----------|
| Kahoku | *Moina* spp.      | 0.709  | 0.001   | 68        |
|        | Viviparidae sp.   | 0.388  | 0.001   | 35        |
|        | *Simochepalus* spp.| 0.333  | 0.001   | 30        |
|        | *Daphnia* spp.    | 0.274  | 0.013   | 45        |
|        | *Rhaeotanytarsus* sp. | 0.268  | 0.012   | 52        |
| Kitadan| *Cleon dipterum*  | 0.452  | 0.001   | 51        |
|        | Cyprinotinae sp.  | 0.414  | 0.007   | 89        |
|        | *Baetis* sp.      | 0.255  | 0.001   | 23        |

### Table 3. Summary of Canonical Correspondence Analysis of Kahoku and Kitadan

| Variable                        | F   | P   | Variable                        | F   | P   |
|---------------------------------|-----|-----|---------------------------------|-----|-----|
| Area (Kahoku or Kitadan)        | 22.34 | 0.002 | days after planting             | 9.54 | 0.002 |
| days after planting             | 9.54 | 0.002 | water permanence               | 3.15 | 0.002 |
| water permanence               | 3.15 | 0.002 | pH                             | 2.96 | 0.004 |
| pH                             | 2.96 | 0.006 | water depth                    | 2.22 | 0.014 |
| water depth                    | 2.22 | 0.016 | Chlorophyll a                 | 1.89 | 0.04  |
| Chlorophyll a                  | 1.89 | 0.036 |                                |     |      |
Figure 1. A view of Sampling Sites at Kahoku (1A); A view of Sampling Sites at Kitadan (1B)
Figure 2A. Kahoku as modern management

Figure 2B. Kakuma as traditional management of “yatsuta”

Figure 2. Paddy Cultivation Calendar at Kahoku (2A) and at Kitadan (2B)
Figure 3. Dendrogram of Cluster Analysis Based on Sørensen Similarity Index of Animal Assemblages on Each Sampling Areas, KH = Kahoku (Site 1-25), KD = Kitadan (Site 3-20)
Figure 4. Biodiversity Parameters; Taxa Richness, Shannon Wiener Biodiversity ($H'$) Index, and Total Invertebrates Biomass per Sampling Event in Kahoku and Kitadan
Figure 5. Proportion of Desiccation Tolerant Versus Desiccation Sensitive Animal Assembly in Number from May to August at Kahoku (n = 30,735) and Kitadan (n = 4,622)
4. Discussion

Biodiversity was highest in the small valley at Kakuma, where traditional paddy cultivation was conducted in an area that amounted to only 1% of the farmland coverage at Kahoku, where paddy cultivation had been modernized. Desiccation-sensitive aquatic invertebrates were also more frequent at Kakuma. Our CCA identified a rank order of environmental variables that were significantly related to animal assemblage structures in the paddy fields: number of days since transplanting, water permanence, pH, water depth, and chlorophyll a concentration. The effects of these variables were also significant in our partial CCA, in which permutations were restricted to the respective areas (i.e., no between-area permutations were considered). The variable “number of days since transplanting” represented the development of invertebrate community over time. The other four variables were related to water management in the respective paddy fields; of the four, water permanence was the most influential factor in both areas. We were unable to determine precisely whether the higher animal diversity at Kitadan was better explained by winter flooding or by continual summer irrigation. Nevertheless, we think it likely that continual summer irrigation was the more important factor because of the rapid increases in biodiversity and the proportion of desiccation-sensitive animals at Kitadan during the summer months. Winter flooding may be of little importance in snowy Ishikawa Prefecture, where most of the paddies remained wet beneath the snow during the cold season. We compared
biodiversity in adjacent paddy fields with and without winter flooding practices in another tract of land within this prefecture and found no significant difference between paddies the following summer (Prasetyo and Tuno, unpublished data); this observation supports our tentative identification of summer irrigation as a major determinant of animal diversity.

Water permanence was important for the development of diversity in the faunal assemblages. Diversity was initially low at Kitadan, but increased constantly over time to reach a plateau, whereas diversity at Kahoku fluctuated temporally, possibly because of the disturbances associated with intermittent irrigation practices. Mogi (1993) reported that intermittent irrigation in paddy fields decreases the abundance and richness of aquatic animals.

Our cluster analysis based on the Sørensen similarity index and our indicator species analysis showed that the two areas (Kahoku and Kitadan) contained area-specific animal assemblages. Based on the indicator values, the Cladoceran *Moina* spp. was the most area-specific taxon at Kahoku, and the Ephemeropteran *Cloeon dipterum* was the most area-specific taxon at Kitadan. *Moina* spp. are common early colonizers and are characteristic of temporary water bodies, probably because they are desiccation tolerant and able to grow rapidly after irrigation (Williams, 2006). *Moina* spp. accounted for 40.9% of the total biomass at Kahoku. Among the insects, the hemipteran *Micronecta* sp. dominated abundance estimates at Kahoku, whereas *Cloeon dipterum* was dominant at Kitadan. *Micronecta* sp. has wings that allow it to escape unfavorable conditions, but this is not the case for the larval stage of *C. dipterum*. Jeffries (2005) reported that *C. dipterum* is associated with water bodies with high water permanence values.

We found that characteristic early colonizers in paddy fields of both areas were desiccation tolerant, but these were gradually replaced by desiccation-sensitive animals as the water persisted in the fields. Protracted water permanence may provide nutrients (Lawler & Dritz, 2005) and extended habitat availabilities for aquatic invertebrates, thereby promoting biodiversity and species richness (Collinson et al., 1995; Tarr et al., 2005; Boulton, 2003). However, our two study areas differed not only in terms of water management practices but also in (i) land use in the tracts surrounding the fields, (ii) the extent of agrochemical usage, and (iii) water irrigation systems, which we did not evaluate in this study. Future studies should be undertaken to determine the influence of these additional factors.

In summary, our study strongly demonstrated that high biodiversity can be attained in small paddy fields where volunteers keep up traditional maintenance practices. However, these procedures have fallen into general disuse because of the hard labor required to produce a small crop. The promotion of biodiversity by traditional agriculture must be weighed against the disadvantage of low crop yield in planning the future of paddy farming in Japan.

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