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ORIGINAL RESEARCH

Using stable isotopes to investigate movement of fish in rice paddy fields

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Abstract We evaluated movement of fish, especially *Misgurnus* spp. (loach), in paddy fields and irrigation ponds by conducting an inventory of $\delta^{13}C$ and $\delta^{15}N$ of fish, potential food sources, and soil organic matter in two irrigation pond–paddy field systems in Korea. The pond–paddy systems differ with respect to the presence or the absence of a ridge between the paddy field and the irrigation pond and also whether or not livestock are present in their watersheds. The ridge prevents the free movement of fish between paddy field and irrigation pond habitats in one of the pond–paddy watersheds, but not in the other watershed. We found differences in loach $\delta^{13}C$ and $\delta^{15}N$ inhabiting the paddy fields compared to those in loach $\delta^{13}C$ and $\delta^{15}N$ in the irrigation ponds. In irrigation ponds, loach $\delta^{13}C$ was lower in September (average −27.9 and −27.7 ‰) compared to July (average −26.2 and −26.3 ‰) in the watershed with a ridge (station 1) and without a ridge (station 6), respectively. Loach $\delta^{13}C$ in irrigation ponds in September was similar to loach $\delta^{13}C$ in the paddy field in July at both sampling sites, indicating loach might have moved into irrigation pond from paddy field. Loach $\delta^{15}N$ in the watershed with livestock was significantly higher (average 18.2 ‰) in the irrigation ponds than loach $\delta^{15}N$ in the watershed with no livestock present (average 11.3 ‰), probably reflecting higher anthropogenic nitrogen inputs from livestock. Differences in $\delta^{13}C$ and $\delta^{15}N$ in the loach reflected changes in habitat utilization of loach between paddy fields and irrigation ponds. Aquatic insect $\delta^{13}C$ differed from loach $\delta^{13}C$ but were more similar to carp $\delta^{13}C$. The stable isotope inventory approach used in this study could be used to augment or replace a more traditional field based mark–recapture approach.

Keywords Fish habitat · Irrigation pond · Misgurnus spp. · Paddy field · Stable isotopes

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Introduction

The inclusion of paddy fields as wetlands in the list of protected Ramsar Convention sites has led to a focus on restoration and maintenance of biodiversity in these agricultural landscapes, including rice paddy fields in Korea (Yamazaki et al. 2004; Kim et al. 2006a, b). Throughout east-Asia, paddy fields are recognized as important landscape features related to biodiversity of wetlands. Freshwater fish populations in paddy fields are a food resource, help control insect pests, and contribute to soil fertility in paddy fields (Vromant and Chau 2005; Aditya et al. 2010; Chen et al. 2013; Kim et al. 2011).

Typically, rice paddy fields in Korea are tilled between April and June and are irrigated prior to planting. Paddy fields are supplied with water from spring to early summer, irrigated intermittently after a short drainage period in summer, and drained in autumn. These paddy fields, together with the irrigation ponds, provide habitats for aquatic insects, amphibians, and fish (Hata 2002; Fujimoto et al. 2008). In particular, *Misgurnus* spp. (loach) is an important commercial and recreational fishery in Korea which is widely distributed in streams and ponds draining agricultural lands. Loach is often among the dominant fish species because loach can thrive in habitats with partial desiccation, low predation pressure, and low dissolved oxygen concentrations (Harding et al. 2007).

One typical field-based method of estimating population size and identifying movement of fish is the mark–recapture method (Pine et al. 2003). However, mark–recapture methods require tagging individuals and can be labor intensive because several repeated sampling periods may be needed. Other approaches to investigate the movement of fish in aquatic ecosystems utilize carbon and nitrogen stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). Stable isotope measurements have been used to study fish migration between freshwater and estuarine habitats (Cunjak et al. 2005). Stable isotopes are especially useful as tracers of animal movement between habitats because $\delta^{13}C$ can trace energy and carbon sources and $\delta^{15}N$ can identify feeding relationships and trophic position (Vinagre et al. 2008). If a stable isotope approach can be shown to be as effective as mark–recapture methods at identifying movement and understanding fish life-history, then incorporating less field-intensive stable isotope approaches in future studies on fish movement in rice paddy field ecosystems would be desirable.

The aim of this study was to use patterns in carbon and nitrogen stable isotope composition of loach (*Misgurnus* spp.) to determine whether loach move from paddy fields to irrigation ponds during the autumn drainage period. These type of studies are needed in Korea and the region to improve our understanding of the importance of small irrigation ponds in supporting biodiversity in agricultural landscapes. This study can also provide important fundamental data on patterns in carbon and nitrogen stable isotope composition in rice paddy field ecosystems in the east-Asia region.

Materials and methods

Study sites

This study was conducted in two paddy fields with irrigation ponds located in Chungcheongnam Province, Korea (Fig. 1). Samples were collected during two seasons (July and September) during 2011. At both study sites, irrigation ponds are surrounded by rice paddy fields, but only the irrigation pond at station 1 is perennial and is characterized by a drainage channel through the adjacent paddy fields. At station 6, the water in the irrigation pond is supplied from an upper paddy field. The pond surface areas are about 14.1 and 11.4 m^2 at stations 1 and 6, respectively. The average water depths are approximately 163 cm at station 1 and 30 cm at station 6. Both paddy fields are fertilized with organic compost during the rice-production season. Grazing by livestock occurs within the station 1 watershed but not in the station 6 watershed.

The dominant fish species in the irrigation ponds are loach (*Misgurnus* spp.), but carp (*Carassius auratus*) can only be found at station 1. There is a ridge between the paddy field and irrigation pond at station 1, but at station 6, the ridge is lacking. At station 6, loach can freely move between habitats (paddy field and irrigation pond; Fig. 1).

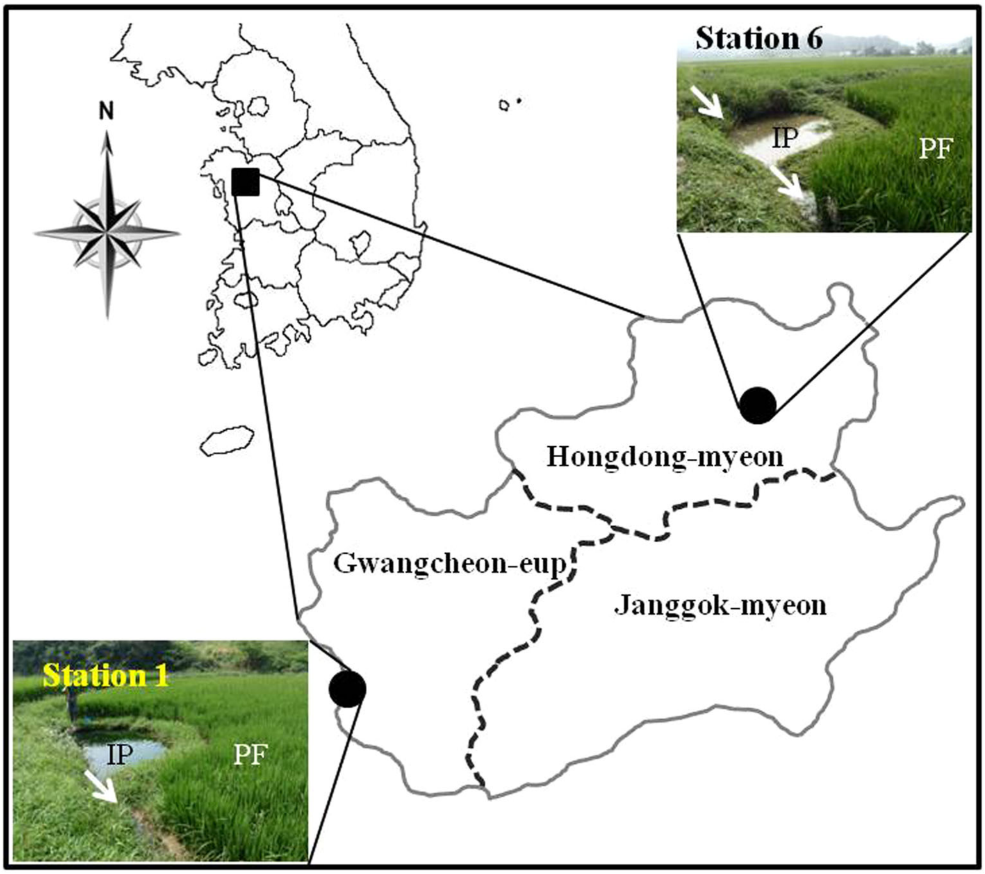


Fig. 1 Location of study sites (station 1, 6). *Arrow* represents direction of water flow. *IP* and *PF* refer to irrigation pond and paddy field

Methods

Fish were sampled using a kick net (width, 1.8 m; mesh size 4 mm) and fish traps (length 25 cm; ring length, 1.5 m; mesh size 5 mm) in the irrigation pond and paddy field. Carp were found only in the perennial irrigation pond at station 1. Macroinvertebrates in the rice paddy fields were collected using a D-frame aquatic insect net (40-cm width; mesh size 1 mm). Water samples were analyzed for concentrations of total nitrogen and total phosphorus measured according to American Public Health Association (APHA) methods (APHA 1998). Water samples for Chl-*a* determination were filtered (GF/F) and processed using 90 % acetone, and Chl-*a* concentration was measured according to standard methods (APHA 1998).

Soil organic matter was collected with a hand trowel (depth 5 cm) from three representative sites adjacent to each irrigation pond. In the laboratory, soil organic matter samples and samples for aquatic insects such as chironomids and *Tubifex* spp. were sieved using standard sieves (mesh size 250 µm).

Samples for carbon stable isotope analysis were acid fumed in a dessicator (1 N HCl for 24 h) to remove inorganic carbon, rinsed in deionized water, and finally dried at 45 °C. Samples for nitrogen isotopic measurement were not acid treated to avoid any treatment effects (Carabel et al. 2006). Samples were loaded into tin capsules, and C and N isotopes were measured using an IsoPrime isotope ratio mass spectrometer (Manchester, UK). The natural abundances of ^{13}C and ^{15}N are expressed as the per mil (‰) difference from the international standard as $\delta^{13}C$ and $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 10^3$ where $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$ and Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 as standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. The analytic variations for replicate measurements were typically better than ±0.3 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope compositions were compared using Student's *t* tests to test for a significant difference between the two sites using a significance level of 0.05 (Zar 1999).

Table 1 Physical and chemical characteristics of irrigation ponds

	Station 1	Station 6
Location	36°31′16.6″N	36°32′21.2″N
	126°38′24.3″E	126°42′02.9″E
Surface area (m^2)	14.1	11.4
Water depth (cm)	163	30
Water temperature (°C)	10.5–17.9 (15.0 ± 2.1)	16.2–28.4 (22.4 ± 4.0)
pH	6.3–8.0 (6.7 ± 0.5)	6.8–9.2 (7.6 ± 0.7)
Conductivity ($\mu S\ cm^{-1}$)	484–829 (601 ± 122)	133–570 (384 ± 114)
DO ($mg\ L^{-1}$)	2.7–19.3 (9.0 ± 5.5)	0.5–14.3 (6.4 ± 4.1)
TP ($mg\ L^{-1}$)	0.023–0.200 (0.100 ± 0.05)	0.091–0.346 (0.225 ± 0.11)
TN ($mg\ L^{-1}$)	1.09–27.55 (21.1 ± 10.66)	1.61–4.29 (2.51 ± 0.86)
Chl-*a* ($\mu g\ L^{-1}$)	1.0–43.3 (15.0 ± 16.0)	4.2–310.0 (82.8 ± 126.5)

WT, DO, TN, TP, and Chl-*a* indicate water temperature, dissolved oxygen, total nitrogen, total phosphorus, and chlorophyll *a* concentrations, respectively ($n = 12$; range and mean ± SD shown)

Results

Characteristics of irrigation ponds

We found some differences in water quality parameters between the irrigation ponds (Table 1). At station 1, TN concentrations were generally higher (average 21.1 mg L^{-1}) compared to station 6 (average 2.5 mg L^{-1}; Table 1). TP in the irrigation pond at station 1 ranged from 0.023 to 0.200 mg L^{-1} (average 0.100 mg L^{-1}); at station 6, TP ranged from 0.091 to 0.346 mg L^{-1} (average 0.225 mg L^{-1}).

Relationship between isotopic composition and total length of fishes

At both stations 1 and 6, loach was present in the paddy field in July; in September, we did not find loach in the paddy fields as the paddy fields were drained. The lengths of loach at station 1 ranged from 62 to 137 mm (average 101.6 ± 27.8 mm, $n = 9$) and from 60 to 113 mm (average 81.2 ± 23.7 mm, $n = 5$), in the irrigation pond and paddy field, respectively (Fig. 2). At station 6, loach varied in length from 42 to 133 mm (average 79.8 ± 30.9 mm, $n = 16$) and from 78 to 113 mm (average 97.5 ± 16.1 mm, $n = 4$) in the irrigation pond and paddy field, respectively (Fig. 2).

The average lengths of loach in the irrigation ponds in September (station 1, 88.2 ± 32.1 mm; station 6, 63.9 ± 19.9 mm) were smaller than the average length in July (station 1, 118.3 ± 3.0 mm; station 6, 106.3 ± 28.3 mm) at both sampling sites (Fig. 2).

In July, loach $\delta^{13}C$ was higher compared to loach $\delta^{13}C$ in September in the irrigation ponds (Fig. 2). Loach $\delta^{13}C$ in September were more similar to the $\delta^{13}C$ of loach in the paddy field in July at both stations. In July, at station 1, loach $\delta^{13}C$ in the irrigation pond ranged from −26.9 to −25.7 ‰ (average −26.2 ± 0.5 ‰). At station 1, loach $\delta^{13}C$ in the paddy field ranged from −28.3 to −26.5 ‰ (average −27.3 ± 0.7 ‰). At station 6, loach $\delta^{13}C$ in the irrigation pond and paddy field in July ranged from −27.4 to −24.5 ‰ (average −26.3 ± 1.1 ‰), and −28.1 to −26.8 ‰ (average −27.4 ± 0.5 ‰), respectively. In September, loach $\delta^{13}C$ in the irrigation ponds in station 1 and station 6 ranged from −29.5 to −27.1 ‰ (average −27.9 ± 0.9 ‰), and −28.4 to −26.6 ‰ (average −27.7 ± 0.5 ‰), respectively.

In July, irrigation pond loach $\delta^{15}N$ was significantly higher than paddy field loach $\delta^{15}N$ in station 1, but not at station 6 (station 1, $P = 0.006$; station 6, $P = 0.078$). However, in September, irrigation pond loach $\delta^{15}N$ was similar to loach $\delta^{15}N$ in the paddy field in July in both stations (Fig. 3; station 1, $P = 0.786$; station 6, $P = 0.476$). In July, irrigation pond loach $\delta^{15}N$ and paddy field loach $\delta^{15}N$ at station 1 ranged from 18.9 to 21.0 ‰ (average 20.2 ± 0.9 ‰) and from 15.4 to 18.3 ‰ (average 17.0 ± 1.5 ‰), respectively. At station 6 in July, irrigation pond loach $\delta^{15}N$ and paddy field loach $\delta^{15}N$ ranged from 9.7 to 18.5 ‰ (average 13.9 ± 4.0 ‰) and 9.7–10.4 ‰ (average 10.2 ± 0.4 ‰), respectively. In September, irrigation pond loach

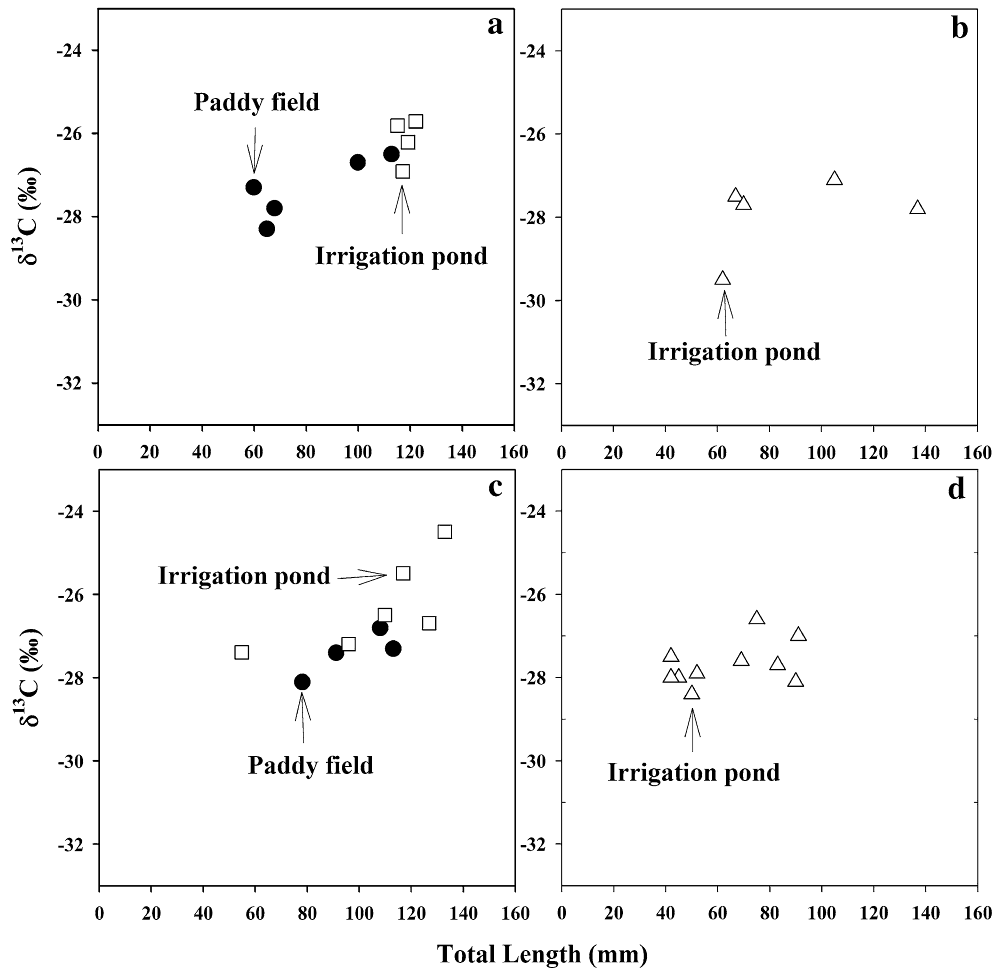


Fig. 2 Relationship between length and $\delta^{13}C$ for *Misgurnus* spp. (loach). **a** Station 1, July 2011; **b** station 1, September 2011; **c** station 6, July 2011; **d** station 6, September 2011

$\delta^{15}N$ at station 1 and station 6 varied from 14.0 to 18.7 ‰ (average 16.7 ± 2.0 ‰) and from 8.2 to 14.6 ‰ (average 9.8 ± 1.9 ‰), respectively.

Carp were only found in the irrigation pond at station 1. The length of carp varied from 26 to 79 mm (average 48.1 ± 22.0 mm, $n = 7$) in July. In September, the length of carp ranged from 21 to 79 mm (average 41.8 ± 17.6 mm, $n = 15$) (Fig. 4).

At station 1, carp $\delta^{13}C$ and $\delta^{15}N$ did not differ between July and September ($P = 0.481$ and $P = 0.795$), respectively. Overall, in the irrigation pond at station 1, carp $\delta^{13}C$ in July and September ranged from −32.0 to −27.9 ‰ (average −30.3 ± 1.7 ‰) and from −33.8 to −28.6 ‰ (average −29.9 ± 1.3 ‰), respectively (Fig. 4). In the irrigation pond at station 1, carp $\delta^{15}N$ in July and September ranged from 15.0 to 17.9 ‰ (average 16.6 ± 0.9 ‰) and from 15.0 to 17.9 ‰ (average 16.6 ± 0.9 ‰), respectively (Fig. 4).

$\delta^{13}C$ and $\delta^{15}N$ in food web components

We examined $\delta^{13}C$ and $\delta^{15}N$ of potential food sources for fish at stations 1 and 6 (Fig. 5). The bivariate plots show that the potential food sources for loach and carp were Tubifex worms (*Tubifex* spp.) and chironomids, respectively. Aquatic insect $\delta^{13}C$ was not clustered near the loach, but at station 1, carp $\delta^{13}C$ was similar to aquatic insect $\delta^{13}C$ (Fig. 5). Carp $\delta^{15}N$ was higher (average 16.3 ‰) than aquatic insect $\delta^{15}N$ (average 11.0 ‰).

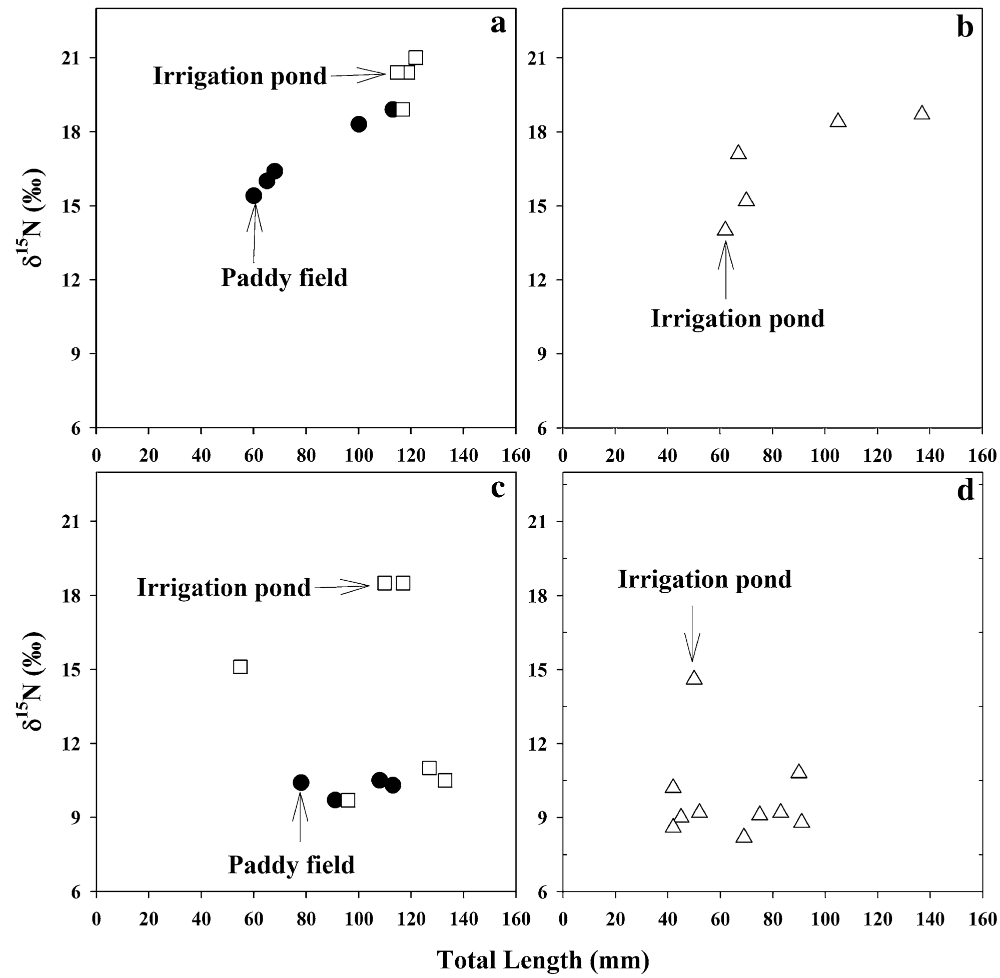


Fig. 3 Relationship between length and $\delta^{15}N$ for *Misgurnus* spp. (loach). **a** Station 1, July 2011; **b** station 1, September 2011; **c** station 6, July 2011; **d** station 6, September 2011

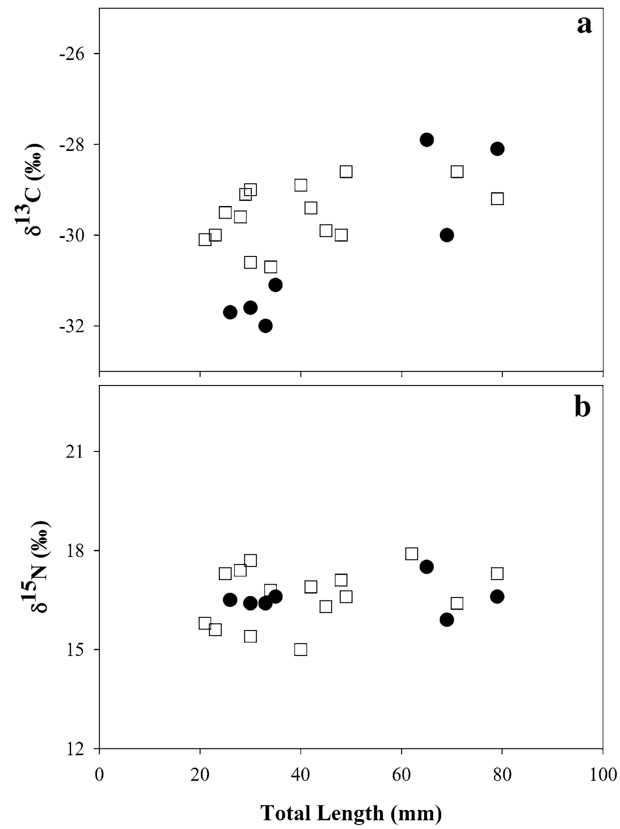
Discussion

Fish movement and hydrologic connectivity

Loach can readily move between different habitats (irrigation pond, connecting channel) according to hydrologic conditions and when irrigation water is drained from paddy fields. The difference in the hydrologic connection (channel width and height) between the two stations is important because there is no ridge between the irrigation pond and paddy field at station 6, allowing fish to move between habitats. However, at station 1, there is a ridge between the irrigation pond and paddy field. Thus, fish movement is influenced by the connectivity in addition to the drainage of standing water from paddy fields.

Other studies have used stable isotopes to identify patterns in fish migration between different habitats (Grey 2001; Maruyama et al. 2001; Kim et al. 2011). In our study, the difference in loach $\delta^{13}C$ between the irrigation pond and paddy field at station 1 (Fig. 2) shows the possibility to use detailed $\delta^{13}C$ measurements of fish and other food web components to study fish movement even at the scale of these small irrigation ponds and paddy field ecosystems. In July, the pattern in loach $\delta^{13}C$ likely reflected the difference in diet for loach living in the paddy fields and loach living in the irrigation pond. In September, regardless of size, loach $\delta^{13}C$ in the irrigation ponds were similar to loach $\delta^{13}C$ in paddy field in July (Fig. 2), indicating that loach changed their habitat from the paddy field to the irrigation pond at station 1. Loach in the paddy field at station 1 cannot move to the irrigation pond until drainage of standing water in paddy field because there is the ridge between the irrigation pond and paddy field. However, at station 6, there is an open connection between the irrigation

Fig. 4 Relationship between length and stable isotope composition for *Carassius auratus* (carp) at station 1. **a** Carp $\delta^{13}C$ and total length (mm); **b** Carp $\delta^{15}N$ and total length (mm) at station 1. *Filled circles* indicate July and *open squares* indicate September



pond and paddy field. Therefore, small-size loach can easily move from the paddy field to the irrigation pond, but large loach may not be able to inhabit shallow water depths for overwintering. The spawning season of the loach extends from mid-May to August (Fujimoto et al. 2008). In these irrigation ponds, small size loach had spawned before the water was drained from the paddy fields, and a number of larger size loach might have moved into the irrigation pond or downstream through a channel (Kim et al. 2011).

We found only minor variation in loach $\delta^{15}N$ with fish length (Fig. 3), suggesting that loach used similar food sources among all size classes. Previous studies have found positive relationships between $\delta^{15}N$ and total length regardless of taxa. The isotopic composition of fish feeding in different habitats can vary with their foraging behaviors and food sources (Yoshioka et al. 1994; Maruyama et al. 2001; Cocheret de la Moriniere et al. 2003). A change in isotopic composition can result from ontogenetic shifts and will reflect trophic relationships among species if, during distinct developmental stages, different food sources are exploited.

Carp $\delta^{15}N$ did not show a difference between July and September, unlike for carp $\delta^{13}C$, indicating little variation in food source $\delta^{15}N$ for carp regardless of body size. The pattern in loach $\delta^{15}N$ seems more related to movement with drainage water to the irrigation ponds or into the channel adjacent to the paddy fields. Loach $\delta^{15}N$ was lower in September than in July, and loach $\delta^{15}N$ in the irrigation ponds in September was similar to paddy field loach $\delta^{15}N$ in July, reflecting that loach in the irrigation pond in September had likely migrated from the paddy field. In these paddy fields and irrigation ponds, fish movement is also related to habitat selection for overwintering during the dry season, emphasizing that even small irrigation ponds provide important habitat for overwintering of fish in these agricultural landscapes.

$\delta^{15}N$ and watershed characteristics

The pattern for loach, aquatic insects, and soil organic matter $\delta^{15}N$ in the two sampling sites (station 1 and station 6) might be related to the difference in land use in the watersheds (Fig. 5). The irrigation pond at station 1 is impacted by livestock in the surrounding watershed and had higher nitrogen concentrations in the irrigation pond. At station 6, there are no livestock grazing in the watershed, and nitrogen concentrations in the

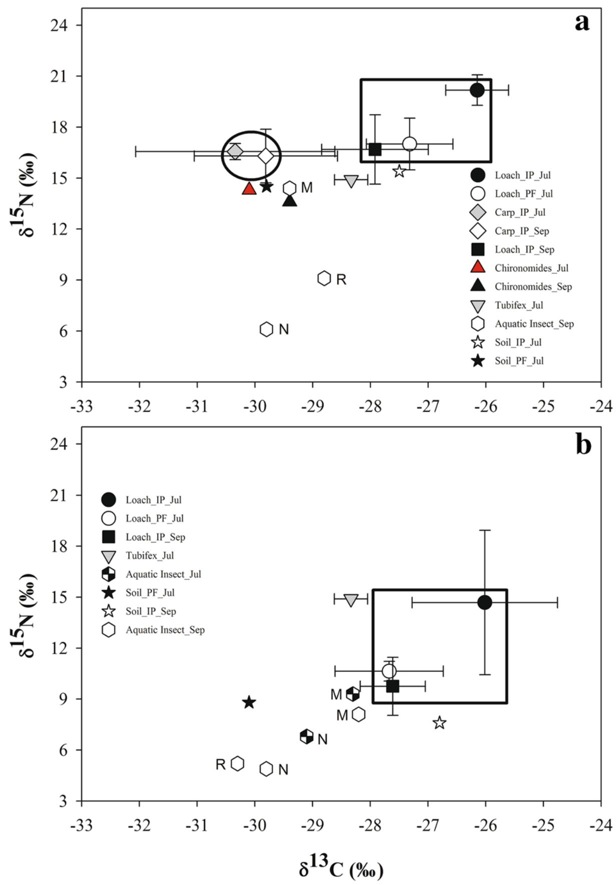


Fig. 5 Bivariate plot showing $\delta^{13}C$ and $\delta^{15}N$ of food web components at station 1 and station 6. *IP* indicates the irrigation pond, and *PF* indicates the paddy field as shown in the legend. **a** station 1; **b** station 6. *Vertical* and *horizontal bars* indicate ± 1 SD. The *boxed area* indicates *Misgurnus* spp. (loach); the *circled area* indicates *Carassius auratus* (carp). Aquatic insects included *Muljarus japonicus* (giant water bug), *Notonecta triguttata* (backswimmer), *Ranatra chinensis* (water stick insect)

irrigation pond were generally lower. Unlike for $\delta^{13}C$, we found a significant difference (about 6 ‰) in loach $\delta^{15}N$ between the sampling stations (Fig. 5). Overall, our results show higher $\delta^{15}N$ for loach and aquatic insects at station 1 compared to station 6 (Fig. 5). The higher $\delta^{15}N$ in these aquatic organisms at station 1 might reflect higher nitrogen inputs from the watershed at station 1. With higher N inputs from livestock manure, increased nitrate, ammonium, or total N concentrations in surface water or groundwater could lead to increased $\delta^{15}N$ of food sources, and fish tissue would have higher $\delta^{15}N$ in the food web (Anderson and Cabana 2006).

Studies in other regions have shown that differences in $\delta^{15}N$ of fauna can reflect anthropogenic N loading to aquatic ecosystems. $\delta^{15}N$ of aquatic consumers can generally be used as an indicator of the magnitude of anthropogenic N loading from watersheds, but food webs in Korea have not been thoroughly studied using stable isotopic methods. In these paddy field ecosystems, the higher $\delta^{15}N$ at station 1 might reflect higher rates of one or more specific mechanisms of nitrogen loss from the system, such as denitrification, a process which results in a large isotopic fractionation (Wada et al. 1984; Diebel and Vander Zanden 2009).

In July, carp $\delta^{13}C$ differed from loach $\delta^{13}C$ in station 1. In September, loach $\delta^{13}C$ and $\delta^{15}N$ were lower at both stations (Fig. 5). The bivariate $\delta^{13}C$ versus $\delta^{15}N$ plot clearly shows a difference in $\delta^{15}N$ between loach and carp at station 1 (Fig. 5). Loach $\delta^{13}C$ was similar to Tubifex worm $\delta^{13}C$ but were less similar to chironomid $\delta^{13}C$. Using stomach content analysis, loach have been shown to consume chironomids, zooplankton, protozoa, and macroinvertebrates (Nakamura and Oda 2003; Kim et al. 2011). The majority of fish species in paddy fields can consume zooplankton and aquatic insects (Knight et al. 2003). Though our study did not identify specific food sources (zooplankton, insects, protozoa), the preliminary patterns we identified in this study show that there is a clear evidence for a difference in $\delta^{13}C$ between carp and loach in these irrigation ponds. The lack of detailed studies of patterns in $\delta^{13}C$ and $\delta^{15}N$ in these irrigation pond and paddy field systems highlights the need for future studies using stable isotopes in paddy field food webs within this important agricultural landscape.

This also suggests that immigrant individuals might be distinguishable between these sites at this small scale using more detailed $\delta^{15}N$ and $\delta^{13}C$ measurements. For the next phase of this research, a study comparing results from mark–recapture methods to quantify fish movement with the stable isotope approach would be valuable.

The presence or the absence of a ridge between paddy field and irrigation pond and the depths of irrigation ponds are important factors determining the distribution of fish populations. Even small irrigation ponds in agricultural landscapes with rice paddy fields can play an important role in providing fish habitat. Continued habitat conservation of irrigation ponds is important to sustain resident fish populations and maintain biodiversity in these agricultural landscapes and expanded use of stable isotope inventory approaches will be useful to quantify fish movement in rice paddy field ecosystems.

Conclusion

In agricultural landscapes with paddy fields and irrigation ponds, the movement of fish is affected by hydrologic and structural characteristics (water depth and characteristics of drainage channels) of the system. Differences in $\delta^{13}C$ and $\delta^{15}N$ in the two fish species reflect changes in the habitat utilization of fish between paddy fields and irrigation ponds. The stable isotope approach used here can be used to identify fish movement to irrigation ponds during water drainage from paddy fields and utilization of irrigation ponds for overwintering during the dry season.

The results of this study highlight some of the dynamic nature of habitat utilization occurring even in small irrigation ponds and paddy fields within rice production agricultural landscapes in Korea. This study has also provided some important basic data for understanding patterns in trophic relationships in small irrigation ponds and shows the need for additional studies using stable isotopes to investigate fish life history and habitat utilization in agricultural landscapes in east-Asia. It will be especially be useful to conduct studies comparing results from mark–recapture methods to study fish movement with a more complete stable isotope inventory. Detailed studies will be needed to more completely understand patterns in fish movement and behavior in irrigation ponds and paddy fields, food web structure, and trophic relationships in paddy field ecosystems in these agricultural landscapes.

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