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Characteristics of Macroinvertebrates Food Webs affected by Dry Channel in an Intermittent Stream System of the Echi River in Japan

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ABSTRACT

The purpose of study is to identify trophic pathways from organic matter to macroinvertebrates in terms of the consumer and to characterize the food webs in an intermittent stream system of the Echi River in Japan. The $\delta^{13}C$ values of macroinvertebrates and their potential food sources indicated the scraper (Psephenoides spp., *Ecdyonurus levis*) and collector-gatherer (*Ephemera strigata, Paraleptonphlebia chocolata*) feed on periphyton and POM (particulate organic matter) *in situ*. *Davidius lunatus*, and *Hexatoma* spp., which were identified as predators, may feed upon *Ephemera strigata* and *Stenelmis* larvae, respectively. At station characterized by seepage water, the $\delta^{15}N$ values of *Ecdyonurus levis*, *Lymnaea auricularia*, and *Rhyacophila nigrocephala* larva probably showed relatively lower values according to its diets. Even in homogenous species, the trophic pathways of macroinvertebrates *in situ* exhibited considerable variation; this reflected the trophic pathways from organic matter to the consumer depending on habitat characteristics in stream.

Key Words: *Functional Feeding Groups, Potential Food Sources, Trophic Pathway*

1. INTRODUCTION

Lotic food webs mainly derive their energy from allochthonous and autochthonous (algae) organic sources (e.g. Vannote *et al.*, 1980; Thorp and Delong, 1994; Ishikawa *et al.*, 2010). Food webs in adjacent habitats and ecosystems may be strongly linked by fluxes of nutrients, detritus, or organisms (Polis *et al.*, 1997). The spatial and temporal scales of connections between food webs were known only fragmentary knowledge. Such information is crucial to understanding the processes that control the dynamics of local populations, communities, and ecosystems, as well as the fate of fluxes of materials and individuals across habitat, ecosystem, and landscape boundaries (Polis *et al.*, 1997; Laurance *et al.*, 2001). The mobility of limiting nutrients, organic matter, and organism characteristic of lotic environments suggests that such linkages are especially strong in river food webs. For example, detrital carbon inputs from terrestrial to stream food webs are important in many stream ecosystems (Hynes, 1975). Organic carbon sources for lotic food webs may be derived from multiple sources, including local algal production and particulate and dissolved organic matter transported from upstream habitats. Consequently, the spatial scales of trophic interactions in rivers are largely unknown (Cooper *et al.*, 1997). Furthermore, relatively few studies have been conducted on food webs related to spring ecosystems. Spring streams are closed ecosystems and arise from seepage areas; in addition, primary production is relatively high in these small spring streams, which are exposed to sunlight, algae-rich, and contain adequate concentrations of nitrogen and phosphate (Allan, 1995).

The aims of this study were to identify the trophic pathways from organic matter to macroinvertebrates in terms of consumers, using stable isotopes (carbon and nitrogen); and to characterize the food webs in an intermittent stream system of the Echi

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River in Japan.

2. MATERIALS AND METHODS

2.1 Study Stations

The Echi River (35°0′N, 136°0′E), one of the major rivers flowing into Lake Biwa, is 50 km long and has a catchment area of approximately 204 km^2 (Fig. 1b). For land use, the proportion of forest occupied more than 80% of the river basins, and those of paddy fields and other farmland are 8.5%, and 0.5%, respectively (these values were calculated using GIS; Yamada *et al.*, 2011). The upstream area is a steep mountainous area while the downstream area is flat land. During the dry season, the Echi River dries up in an area located 11~17 km from the river mouth (Fig. 1c). We selected four random sampling stations along the river. Marked seepage was observed at the end of the dry channel. We established four stations (ES: Echi River Station; i.e. ES 1, ES 2, ES 3, and ES 4) according to horizontal distribution. This study was conducted in two stations upstream (ES 1 and ES 2) and two stations downstream (ES 3 and ES 4) of the dry channel area in the Echi River (Fig. 1).

In terms of the general environmental parameters, WT and pH concentrations were greatly higher at ES 4 (28℃, pH 8.6) than at ES 1~ES 3 (24~25℃, pH 7.1~7.2), DO concentrations at ES 3 (6.5 mgL^{-1}) were relatively lower than those at the other stations (9.0~9.9 mgL^{-1})(Shin *et al.*, 2013).

2.2 Sample Collection and Preparation

Benthic macroinvertebrates were collected at the 4 stations in August 2008. We conducted sampling with three replicates, using a square Surber net sampler (250 μm mesh; size, 0.25 m^2). All biota were separated from other material and sorted in the laboratory. Identification was mostly performed using a stereoscopic microscope at 10~40×magnification.

Macroinvertebrates were identified primarily at the species and genus level. Among the collections from sampling stations, common taxa were analyzed and distributed widely in order to provide a better comparison at each station. The taxa included Psephenoides spp., *Ecdyonurus levis*, *Ephemera strigata*, *Davidius lunatus*, *Lymnaea auricularia*, *Paraleptophlebia chocolata*, *Stenelmis* spp., *Rhyacophila nigrocephala*, *Hexatoma* spp. Based on their relatively high abundance, these macroinvertebrates were selected for the following analyses.

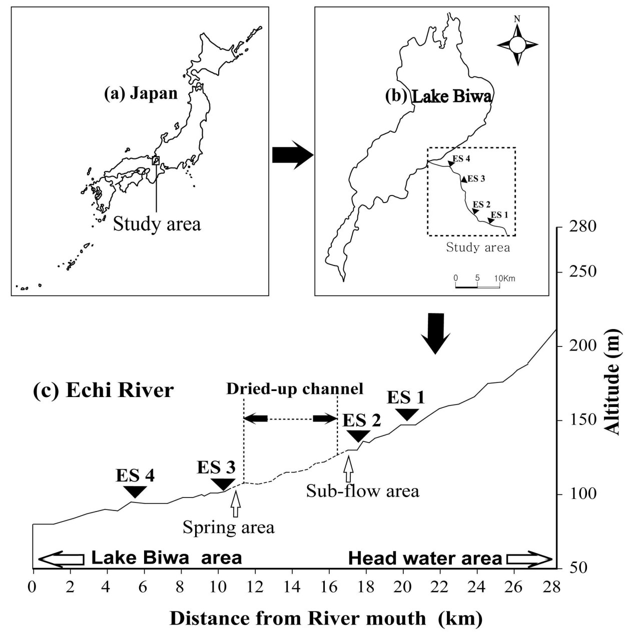

Fig. 1. (a) Map of the study area in Japan, (b) Map of the study stations along the Echi River, (c) Longitudinal section of the Echi River. ES 1 and ES 2 are upstream, while ES 3 and ES 4 are downstream of the dry channel in the river (modified by Shin et al., 2013).

2.3 Analysis of Stable Isotope Ratios

Macroinvertebrates were collected from all of the sampling stations. The macroinvertebrates were maintained in filtered river water at 5℃ for 24 h to allow them to eliminate their gut contents. The samples were defatted in a solution of chloroform and methanol (2:1) according to the method of Folch *et al.* (1957). Defatting was carried out in order to avoid variations in the $\delta^{13}C$ because of the differences according to species in the concentration of isotopically lighter lipids (Focken and Becker, 1998), and the samples were then freeze-dried and stored in a freezer at −20℃ until the stable isotope ratios were analyzed.

The potential food sources included suspended particulate organic matter (SPOM), benthic particulate organic matter (BPOM), and periphyton. For the analysis of stable isotopes, SPOM was collected by filtering the river water through GF/F glass fiber filters, BPOM was collected from the streambed using sieves with mesh sizes: <250 μm, and periphyton was collected by scouring the surface of stones with quadrat (5 cm × 5 cm) using a brush. SPOM, BPOM and periphyton samples were acidified with 1 N HCl to remove carbonates. All the samples were freeze-dried and stored in a freezer at −20℃ until the stable isotope ratios were analyzed.

Three replicate measurements of the carbon and nitrogen isotope ratios were taken for each sample. Generally 2 to 250 individuals of the same species were compiled in each sample. The value of all species were measured as an average of compiled individuals (Psephenoides spp.: 15～50, *Ecdyonurus levis*: 20～140, *Ephemera strigata*: 60～90, *Davidius lunatus*: 2～3, *Lymnaea auricularia*: 2～4, *Paraleptophlebia chocolate*: 190～250, *Rhyacophila nigrocephala*: 15～30, and *Hexatoma* spp: 6～10 individuals / 1 sample).

The carbon and nitrogen stable isotope ratios of the samples were measured with an elemental analyzer EA1108 (Fisons) connected to a mass spectrometer (Delta S, Finnigan MAT) with an interface (Conflo II, Finnigan MAT) with three replicates. The results are reported using delta notation calculated as: $\delta^{13}C$ or $\delta^{15}N$ (‰) = (R_{sample} / $R_{standard}$ − 1) × 1000. The R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio for $\delta^{13}C$ or $\delta^{15}N$, respectively. The standards for $\delta^{13}C$ or $\delta^{15}N$ were used Pee Dee Belemnite (PDB) and air nitrogen, respectively. The analysis errors for $\delta^{13}C$ or $\delta^{15}N$ were within ± 0.2‰.

Analysis of trophic relationships using stable isotope ratio is generally based on the premise that $\delta^{13}C$ enrichment during trophic transfer is slight (0.8 ± 1.1‰, mean ± SD), whilst that of $\delta^{15}N$ is substantial (3.4 ± 1.1‰) (DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Post, 2002).

3. RESULTS

The mean $\delta^{13}C$ and $\delta^{15}N$ values of macroinvertebrates (ranging from −28.7 to −19.4‰ and 2.3 to 9.9‰, respectively) differed among stations and species (Table 1). The consumers such as Psephenoides spp., *Ecdyonurus levis*, *Ephemera strigata*, *Rhyacophila nigrocephala*, and *Davidius lunatus* were common to both ES 1 and ES 2. The mean $\delta^{13}C$ values of these species showed gradually increasing pattern from ES 1 to ES 2 (Psephenoides spp.: −23.1 to −21.2‰, *Ecdyonurus levis*: −28.7 to −25.1‰, *Ephemera strigata*: −25.6 to −23.3‰, *R. nigrocephala*: −23.8 to −21.1‰, and *Davidius lunatus*: −24.5 to −23.9‰). Although the mean $\delta^{15}N$ values of Psephenoides spp., *Ecdyonurus levis*, and *Ephemera strigata* did not differ among stations (ranging from 2.3 to 2.7‰, 4.5 to 4.9‰, and 5.2 to 5.9‰, respectively), those of *R. nigrocephala* and *Davidius lunatus* displayed increasing from ES 1 to ES 2 (5.4 to 6.9‰ and 5.2 to 6.1‰, respectively). Psephenoides spp. and *R. nigrocephala* were also observed at ES 4. The mean $\delta^{13}C$ values of Psephenoides spp. at ES 2 were relatively higher, whilst those of *R. nigrocephala* at ES 4 were relatively higher than at the other stations.

The $\delta^{13}C$ values of *Ecdyonurus levis* and *Lymnaea auricularia* little differ at ES 2 and ES 3 (ranging from −26.1 to −24.6‰ and −22.0 to −21.7‰, respectively), whilst the mean $\delta^{15}N$ values of these species tended to decrease from ES 2 to ES 3 (4.9 to 2.5‰ and 5.6 to 2.5‰, respectively). The $\delta^{13}C$ and $\delta^{15}N$ values of *Paraleptophlebia chocolata* showed increasing pattern from ES 3 to ES 4 (−23.9 to −19.4‰ and 3.1 to 6.1‰, respectively). Moreover, the $\delta^{15}N$ values of macroinvertebrates at ES 3 (ranging from 2.4 to 3.1‰) were relatively lower, whilst those at ES 4 (ranging from 4.2 to 9.9‰) were relatively higher than at the other stations. We used data of Shin *et al.* (2013) in relation to carbon and nitrogen stable isotope ratio for SPOM, BPOM, and periphyton, in order to reveal relationship between macroinvertebrates and their food sources (Fig. 2).

4. DISCUSSION

The potential food sources of macroinvertebrates included suspended particulate organic matter (SPOM), benthic particulate organic matter (BPOM), and periphyton (Allan, 1995). Commonly, even in the mean $\delta^{13}C$ value of 0.8‰ and mean $\delta^{15}N$ value of 3.4‰ in the isotopic fractionation (DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Post, 2002) between macroinvertebrates and their food sources (SPOM, BPOM and periphyton) were considered, our results showed slightly difference between macroinvertebrates and their food sources. In this study, $\delta^{13}C$ value of macroinvertebrates showed more than 5‰ of difference with periphyton of potential food sources

Table 1. $\delta^{13}C$ and $\delta^{15}N$ values of macroinvertebrates at each station in the Echi River

Station	FFGs	Consumer	$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)				*n*
			Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	
ES 1	Scarper	Psephenoides spp.	−23.1	-	−23.3	−22.9	2.3	-	2.2	2.4	2
	Collecter-gatherer	*Ecdyonurus levis*	−28.7	-	-	-	4.5	-	-	-	1
	Collecter-gatherer	*Ephemera strigata*	−25.6	-	-	-	5.2	-	-	-	1
	Predator	*Rhyacophila nigrocephala*	−23.8	-	-	-	5.4	-	-	-	1
	Predator	*Davidius lunatus*	−24.5	-	−24.1	−24.8	5.2	-	5.1	5.4	2
ES 2	Scarper	*Lymnaea auricularia*	−22.0	-	-	-	5.6	-	-	-	1
	Scarper	Psephenoides spp.	−21.2	-	-	-	2.7	-	-	-	1
	Collecter-gatherer	*Ecdyonurus levis*	−25.1	-	−25.6	−24.6	4.9	-	4.9	5.0	2
	Collecter-gatherer	*Ephemera strigata*	−23.3	-	−23.8	−22.8	5.9	-	5.7	6.0	2
	Predator	*Rhyacophila nigrocephala*	−21.1	-	-	-	6.9	-	-	-	1
	Predator	*Davidius lunatus*	−23.9	-	-	-	6.1	-	-	-	1
ES 3	Scarper	*Lymnaea auricularia*	−21.7	-	−22.1	−21.2	2.5	-	2.4	2.7	2
	Collecter-gatherer	*Rhyacophila chocolata*	−23.9	-	-	-	3.1	-	-	-	1
	Collecter-gatherer	*Ecdyonurus levis*	−26.1	-	-	-	2.5	-	-	-	1
ES 4	Scarper	Psephenoides spp.	−20.0	-	−20.1	−20.0	4.3	-	4.2	4.4	2
	Collecter-gatherer	*Rhyacophila chocolata*	−19.4	-	-	-	6.1	-	-	-	1
	Collecter-gatherer	*Stenelmis* spp.	−20.9	-	-	-	7.4	-	-	-	1
	Predator	*Hexatoma* spp.	−20.4	-	-	-	9.9	-	-	-	1
	Predator	*Rhyacophila nigrocephala*	−23.3	1.1	−24.5	−22.6	6.9	0.3	6.6	7.2	3

n indicates the number of the analyzed samples. Blank (-) indicate item that could not be calculated or measured due to a small number of samples. FFGs indicate functional feeding groups. SD indicate standard deviation.

(Fig. 2). The large difference between macroinvertebrates and periphyton can be explained by below reasons.

First, the macroinvertebrates has body with an exoskeleton made of chitin during growth. Lee *et al.* (2002) reported that water mite which sucks only body blood from organisms will assimilate the organic matters the value of which is higher than the whole body of the prey organism. In this study, $\delta^{13}C$ value of macroinvertebrates mainly feeding on periphyton can be lower than those of food sources because chitin is isotopically lighter than muscle in organisms (Schimmelmann and DeNiro, 1986).

Second, Ephemeroptera may have feeding habit as scraper, as well as collector-gatherer according to around food environment (Shin *et al.*, 2011; Merritt and Cummins, 1996).

Relationship between macroinvertebrates and their food sources are shown in Fig. 2. Although the $\delta^{13}C$ and $\delta^{15}N$ values of SPOM and BPOM did not differ between ES 1 and ES 2, those of Psephenoides spp., and *Ephemera strigata* in the upper reaches changed in parallel with those of periphyton *in situ* (Fig. 2). Moreover, the $\delta^{13}C$ and $\delta^{15}N$ values of *Paraleptophlebia chocolata* in the lower reaches were also changed in parallel with those of periphyton. This indicates that these macroinvertebrates assimilated the food sources having much more periphyton than POM. However, the $\delta^{13}C$ and $\delta^{15}N$ values of *Ecdyonurus levis* were close to those POM at each station (ES 1, ES 2, and ES 3). Merritt and Cummins (1996) reported

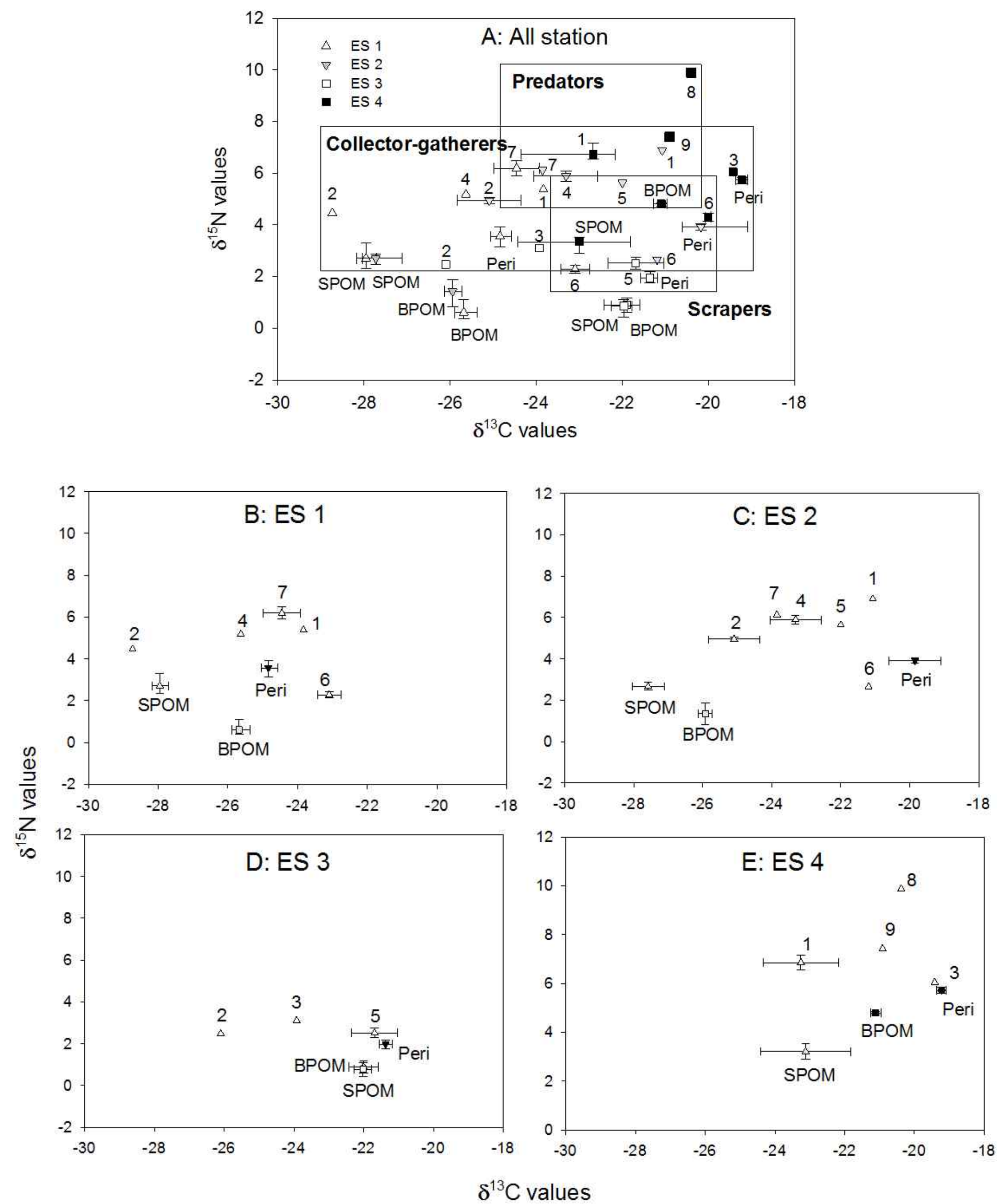

Fig. 2. Mean carbon and nitrogen stable isotope plots of macroinvertebrates and their potential food sources at the respective stations along the Echi River. The square frames indicate the classification of aquatic insects according to functional feeding groups. The open triangles indicate ES 1, gray triangles indicate ES 2, open squares indicate ES 3, and black squares indicate ES 4. SPOM: suspended particulate organic matter, BPOM: benthic particulate organic matter, 1: *Rhyacophila nigrocephala*; 2: *Ecdyonurus levis*; 3: *Paraleptophlebia chocolata*; 4: *Ephemera strigata*; 5: *Lymnaea auricularia*; 6: Psephenoides spp.; 7: *Davidius lunatus*; 8: *Hexatoma* spp.; 9: *Stenelmis* spp. SPOM, BPOM, Periphyton data from Shin *et al.* (2013).

that *Ecdyonurus levis* belong to either the scraper or collector-gatherer group. However, our results indicate that may be closer to the collector-gatherer group than the scraper group. Moreover, they reported that *R. nigrocephala*, *Davidius lunatus*, and *Hexatoma* spp. belongs to the predator group. The $\delta^{13}C$ values of *Davidius lunatus* were similar to, and particularly appeared to change in parallel with, those of *Ephemera strigata*, suggesting that the larvae of *Davidius lunatus* may feed on *Ephemera strigata* in the upper reaches. Furthermore, the $\delta^{13}C$ values of *Hexatoma* spp. at ES 4 were similar to those of *Stenelmis* spp., indicating that the larvae of *Hexatoma* spp. may feed on *Stenelmis* spp. larvae at ES 4. These results were similar to the result of the Inukami River reported by Shin *et al.* (2011), who studied on variation in trophic pathways and food web characteristics in an intermittent stream system. The $\delta^{13}C$ values of macroinvertebrates and periphyton tended to increase from the upstream to the downstream reaches (Fig. 2). They (Shin *et al.*, 2013) reported that the water temperature and pH at ES 4 showed the relatively higher (WT 28.3℃, pH 8.6) than those at the other stations (WT 23.7 to 24.9℃; pH 7.1 to 7.2). Presumably, these results suggested that the enriched $\delta^{13}C$ values at ES 4 reflect a shift from CO_2 to bicarbonate as a substrate for algal photosynthetic activity according to these parameters.

The $\delta^{15}N$ values for *Ecdyonurus* levis and *Lymnaea auricularia* considerably decreased from ES 2 to ES 3 (Table 1). The $\delta^{15}N$ values of their potential food sources at ES 3 ranged from 0.3 to 2.2‰ (Fig. 2), indicating that relatively lower stable isotope signal of *Ecdyonurus levis* and *Lymnaea auricularia* reflected those of its diet *in situ*. In general, denitrification between ES 2 and ES 3 may be show heavy $\delta^{15}N$ values of periphyton and then also macroinvertebrates. However, ES 3 influenced by seepage water showed relatively lower $\delta^{15}N$ values of macroinvertebrates according to its diets. These results suggested that the diminished $\delta^{15}N$ values of the same food sources originated from periphyton, which assimilated by light $\delta^{15}N$ associated with relatively low ammonium concentration (Shin *et al.*, 2013), may directly influence low trophic level of macroinvertebrates. Shin *et al.* (2013) reported that although ES 3 was characterized by groundwater derived from seepage water, the physicochemical parameters did not differ among stations. Probably seepage water would be little influenced by the industrial activities such as the inflow of contaminants from the surrounding watershed (Verbanck *et al.*, 1989), and consequently ES 3 showed relatively lower chloride, sulfate, and sodium ions (Shin *et al.*, 2013).

In conclusion, our results revealed that the macroinvertebrates and periphyton tended to get enriched $\delta^{13}C$ values from the upper to lower reaches in relation to the physicochemical parameters such as water temperature and pH for longitudinal distribution in the Echi River, except for ES 3. Thus, in this study, habitat such as the seepage water region in streams reflects the change in habitat of organisms. The stable isotopic analysis of macroinvertebrates may be use as indicator to reflect stream states in relation to the trophic pathways according to *in situ* food source in the dry or wet stream.

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