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Spatial and Temporal Variation in Stable Isotopes Signatures of Periphyton and an Endangered fish in a flow-reduced River Reach

Huang, W. [1,2*], Yano, S.[1], Zhang, J.M.[3] and Wang, Y. R.[3]

[1]Department of urban and environment engineering, Kyushu University, Fukuoka, 8190395, Japan

[2]China Institute of Water Resources and Hydropower Research, Beijing, 10038, China

[3]State key laboratory of hydraulics and mountain river engineering, Sichuan University, Chengdu, 610065, China

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ABSTRACT: The environmental and ecological issues in the flow-reduced river reach are very serious and are receiving increasingly attention. In this study, we investigate the temporal and spatial changes in $\delta^{13}C$ and $\delta^{15}N$ of Periphyton and isotopic relationship between Periphyton and the endangered fish (Ayu, *Plecoglossus altivelis*) in a flow-reduced river reach in Japan. Much of the spatial and temporal variation in $\delta^{13}C$ of endangered fish was explained by variation in Periphyton, indicating a strong link between primary production and higher order consumers. The Periphyton $\delta^{13}C$ was correlated with current velocity while it was not correlated with the Periphyton biomass (Chl.a). Spatially, the relatively high $\delta^{13}C$ and $\delta^{15}N$ of Periphyton in the lower sites of the flow-reduced river reach might be due to high level of anthropogenically derived N and C introduced from the surrounding agricultural activities and human settings. The trophic position of the endangered fish in was estimated to be 1.5-2.2(1.8±0.3), which agrees with the previous stomach content analysis suggesting that the ayu mainly feed on Periphyton.

Key words: $\delta^{13}C$, $\delta^{15}N$, Periphyton, Endangered fish, Flow-reduced river reach

INTRODUCTION

Since the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of a prey are a reflection of its trophic position and the carbon sources on which it is feeding, respectively (Vander Zanden *et al.,* 1997), stable isotope analysis has been increasingly used to investigate the food web structures in various aquatic ecosystem (Cabana & Rasmussen, 1994; France, 1995; Vander Zanden *et al.,* 1997; Post, 2002). However, application of stable isotope analysis to identify food sources of higher trophic levels in rivers has been plagued by highly variable $\delta^{13}C$ and $\delta^{15}N$ signatures in Periphyton, the main source of autochthonous carbon in most lotic ecosystems (France 1995). Therefore, examination on temporal and spatial variations of stable carbon and nitrogen isotopes in Periphyton and dominant consumers will be important for the understanding of food web structure constructed by stable isotopes.

The present study was conducted in a flow-reduced river reach below a diversion dam from summer to fall in 2006. The aims were to describe the temporal and spatial variations of stable carbon and nitrogen isotopes in Periphyton and an endangered fish, and to describe temporal variations of stable carbon and nitrogen isotopes of the endangered fish (Ayu, *Plecoglossus altivelis*), an endangered fish in this reach, and to discuss the possible mechanisms underlying these variations.

MATERIALS & METHODS

The study reach is located in the upper region of the Ohyama River in Japan (Fig. 1). There are three dams on the river for multipurpose (e.g. food control and hydropower). After completions of the dams, the water was mainly transferred from the lower dam reservoirs to generate hydropower via big pipes (Fig. 1); the flow in the reach is limited to be 1.5m^3/s all over the year. The river ecosystem in this reach (called flow-reduced reach) was severely damaged by the altered flow regime and fragmentation, especially causing the famous fish (Ayu) to be endangered. Sampling of Periphyton was conducted monthly from June to October in 2006. Three sites with comparable physical conditions (e.g. similar, substrata and slope) below the dam (Fig. 1) have been selected to survey. Prior to sampling the Periphyton, the water velocity was measured by using electromagnetic current sensor

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*Corresponding author E-mail: hwfly@yahoo.cn

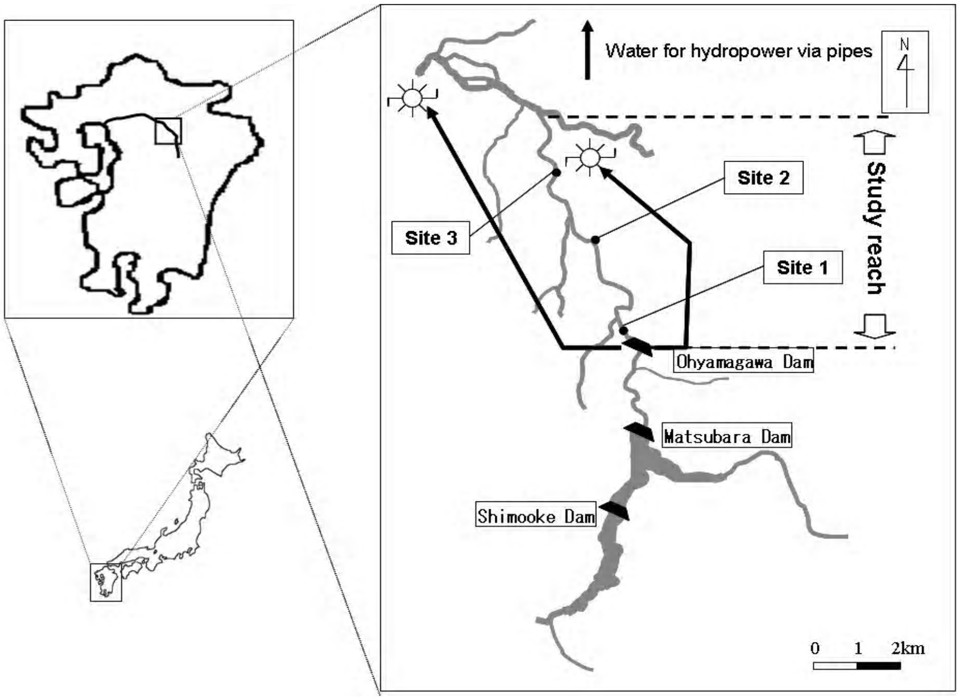

Fig. 1. The study reach and location of sampling sites

(AEM213-D). Periphyton was scrubbed from three to five representative cobbles (10 to 15cm diameter) at from each site. The stones were brushed vigorously at a certain upper area to remove all Periphyton. All the samples were transported to laboratory on ice within 4 hours. In the laboratory, each sample was equally divided into two subsamples for determining biomass (Chlorophyll a) and stable isotope ratios, respectively. Chlorophyll-a was determined by extracting with 90% acetone and steeping in the dark at $4o^C$ for 24h and then measured spectrophotometrically according to APHA (1995). Chlorophyll a concentration was converted to biomass per unit area ($\mu g/cm^2$). For determination of stable isotopes of Periphyton, the subsample were acidified with superfluous 1N HCl solution to dissolve possible calcium carbonate ($CaCO_3$), followed by a rinse in distilled water.

They were then dried to a constant weight by using a freeze-dryer (EYELA FDU-506, Tokyo, Japan). Dried samples were ground to a fine powder with mortar and pestle and stored in clean glass vials for stable isotope analysis. The ayu was collected monthly with the help of fishermen from June to October in 2006. White dorsal muscle of the fish was taken for analysis, since it is less variable in terms of since it is less variable in terms of $\delta^{13}C$ and $\delta^{15}N$ than other tissues (Pinnegar & Polunin, 1999). The fish samples were dried at $60o^C$ to a constant weight, ground to a fine powder and stored in a clean glass vials for stable isotope analysis. Stable carbon and nitrogen isotope ratios were analyzed by using the continuous flow isotope ratio mass spectrometer (ANCA-SL,PDZ Europe). For both Periphyton and tissues, about 0.8 mg sample was prepared for tin capsules. All samples were analyzed two or more times as replicates. Isotopic values were expressed in delta notion (parts per thousand deviation from a standard material), $\delta X = \left[R_{sample} / R_{standard} - 1 \right] \times 1000$; where X is ^{13}C or ^{15}N, $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$, δ is the measure of heavy to light isotope in the sample, whereby higher δ values denote a greater proportion of the heavy isotope. The standard reference materials were Pee Dee Belemnite (PDB) and atmospheric nitrogen for carbon and nitrogen, respectively (Craig, 1957; Mariotti, 1983). The standard deviations of $\delta^{13}C$ and $\delta^{15}N$ replicate analyses were less than 0.2‰ and 0.4‰, respectively. We used t-test to teat for differences. All statistical analyses were run using SPSS software (version 15, SPSS, Chicago, Illinois). Data were analyzed the normality and log-transformed to meet the normality. All statistics were considered significant at the $P < 0.05$ level.

RESULTS & DISCUSSION

Periphyton biomass was expressed in the term of Chl.a ($\mu g/cm^2$). The variations of Periphyton biomass at three sites were shown in the Fig. 2. In July, the Periphyton at the three sites was scoured to low levels as the spate. After the flood, the Periphyton biomass at the three sites recovered rapidly. Their accrual patterns were almost the same. The biomass was higher at the site 1 compared to site 2 and site 3 after the flood.

The $\delta^{13}C$ of Periphyton samples varied monthly during the period (not enough sample in July), ranging from -15.27‰ to -14.09‰ (Fig. 3). The $\delta^{13}C$ of Periphyton samples was negatively correlated to the velocity (Fig. 4). There was obvious relationship between the $\delta^{13}C$ of Periphyton and biomass (Fig. 5). The $\delta^{13}C$ of Ayu showed a similar variation as the Periphyton (Fig. 3). The difference in $\delta^{13}C$ between the Periphyton and Ayu was less than 1‰.

The $\delta^{15}N$ of Periphyton varied from 4.85‰ to 6.28‰ while the $\delta^{15}N$ of Ayu varied from 6.28‰ to 7.69‰ (Fig. 6). The stable isotope values showed a relatively stable variation during the study period. There was no significant correlation between $\delta^{15}N$ of Periphyton and $\delta^{15}N$ of Ayu(r=-0.85, p=0.15).

Both $\delta^{13}C$ and $\delta^{15}N$ values of Periphyton increased from site 1 to site 3 (Fig. 7). There were statistically significant differences in $\delta^{13}C$ among the sites (df=14.81, p<0.001) while there were no statistically significant differences in $\delta^{15}N$ (df=1.28, p=0.326).

Food web is one of the key functional components in river ecosystem (Mercado-Silva, *et al.*, 2009). Understanding the food web, especially the endangered one, can be used to guide conservation of vulnerable species. However, there is rare information on the food web in flow-reduced reach. In the flow-

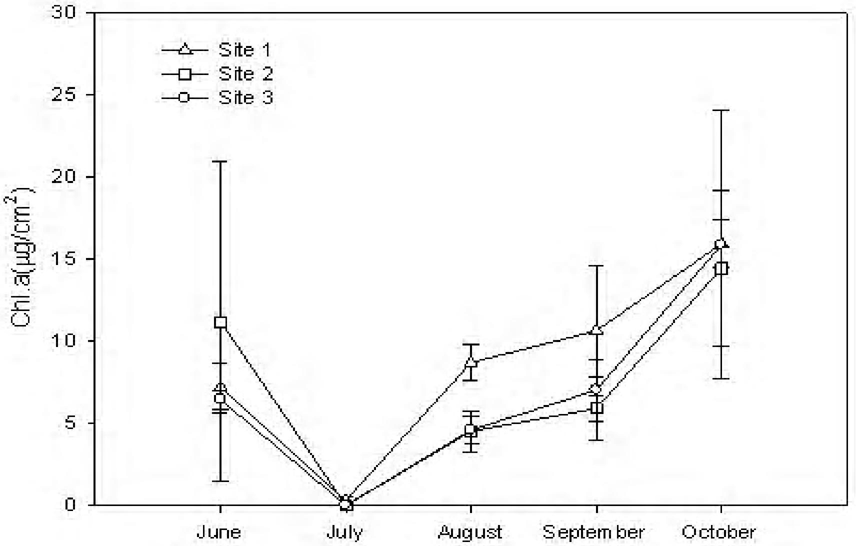

Fig. 2. The Periphyton biomass at the three sites during the study period

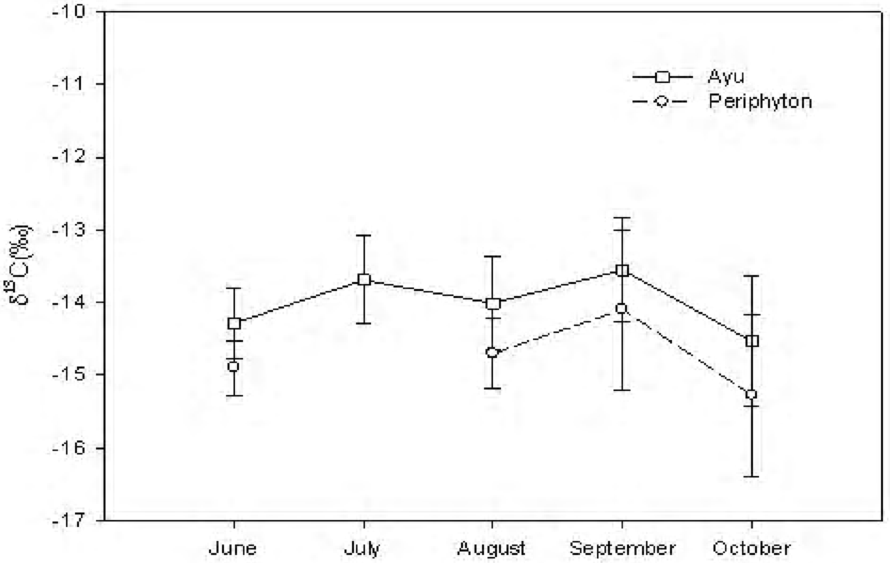

Fig. 3. Monthly variations in $\delta^{13}C$ of Periphyton and Ayu in the reach

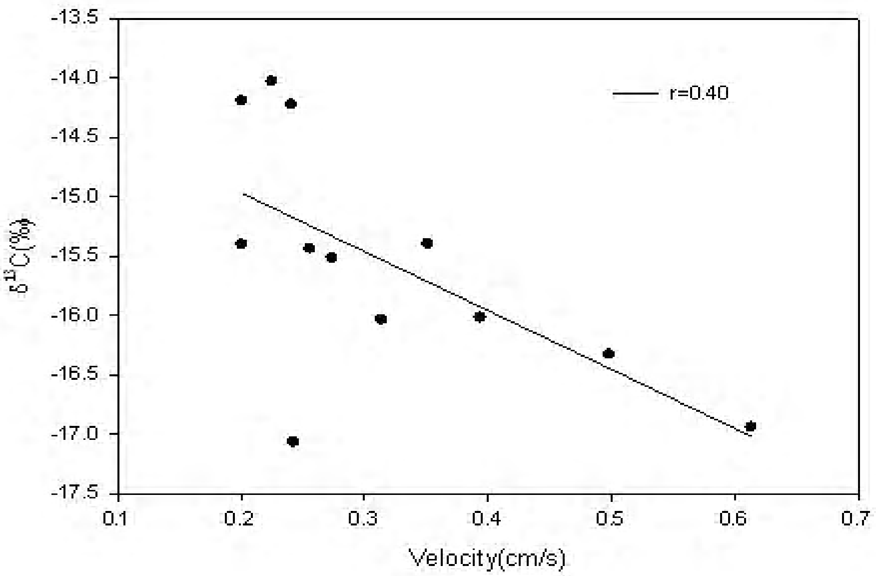

Fig. 4. The relation between Periphyton $\delta^{13}C$ and current velocity

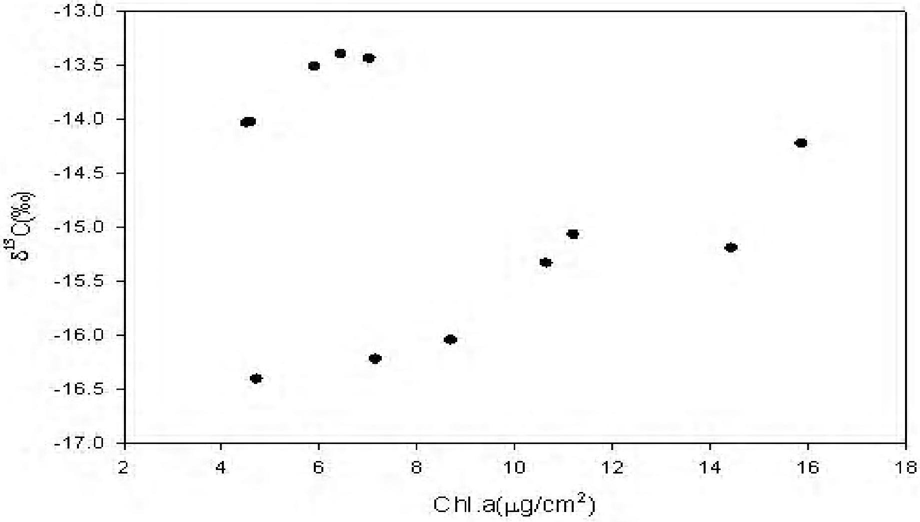

Fig. 5. The relation between Periphyton $\delta^{13}C$ and biomass

reduced reach, the average $\delta^{13}C$ values of Periphyton samples are within the range (-12‰~-48‰) of freshwater Periphyton reported in the literature (Kobayashi *et al.,* 2011). Similar to previous results (Mccutchan & Lewis, 2001), the values in the regulated reach also showed a temporal variation in Periphyton $\delta^{13}C$. However, the values did not show a significant variation. The possible reason is that the conditions are relatively stable in the flow-reduced river reach. The $\delta^{13}C$ value of Periphyton is decreased with increased current velocity in the reach, which is coincided with in some field studies (Finlay *et al.,* 1999; Hill & Middleton, 2006) and laboratory study (Trudeau and Rasmussen, 2003). Finlay al. (1999) suggested that velocity was one of the primary factors that govern carbon isotope fractionation during photosynthesis of Periphyton, because it can affect the thickness of the boundary layer and indirectly affect the DIC (dissolved inorganic carbon, e.g.CO_2) availability for photosynthesis. Previous study(Hill & Middleton, 2006) indicated that the Periphyton $\delta^{13}C$ was positively correlated with the biomass, because the higher biomass often have thicker matrix, which impedes the DIC diffuse into the inner layer of Periphyton matrix and results in relatively high $\delta^{13}C$ of Periphyton. However, in our study, the relationship was only obvious at the site 1. When pool all the data together, there was no correlation between the biomass and Periphyton $\delta^{13}C$. The results might be result from the different habitats, because Hill & Middleton (2006) fount that the relation varied from site to site. The $\delta^{13}C$ of Periphyton increased longitudinally from site 1 and

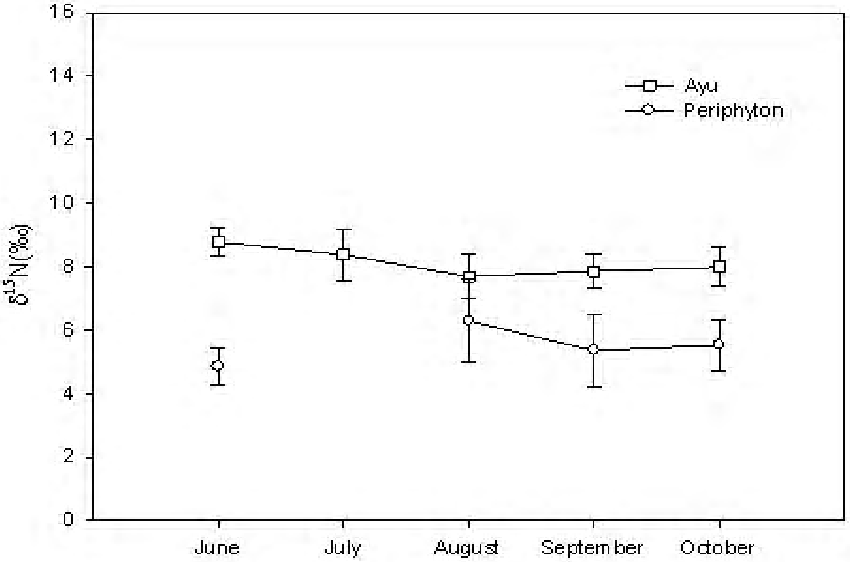

Fig. 6. Monthly variations in $\delta^{15}N$ of Periphyton and Ayu in the reach

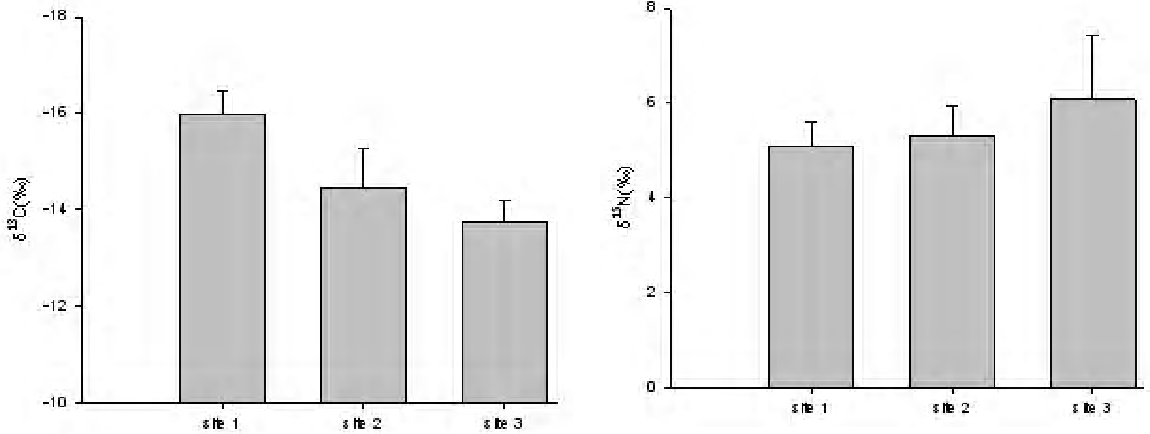

Fig. 7. Spatial variation in $\delta^{13}C$ and $\delta^{15}N$ values of Periphyton at the three sites

site 3(Fig. 7). The result is similar to other results, in which the $\delta^{13}C$ of Periphyton is increased with increasing watershed area (Finlay, 2001) and is increased longitudinally below dams (Winemiller *et al.,* 2011). There are two possible reasons for the results. One is the limitation of the DIC for high productivity as the decreased canopy cover in the downstream area. The other one is the ^{13}C derived from the surrounding human setting and agricultures (Duda, *et al.,* 2011). In our study, we did not measure the DIC longitudinally, but previous the DIC is related to the pH values. The pH values indicated that the DIC is limited in lower sites (unpublished data). However, many factors (e.g. temperature, light, et.) control the mechanism of the $\delta^{13}C$ of Periphyton in river ecosystem and these factors are usually interacted (MacLeod & Barton, 1998), it is difficult to examine the relative importance attributing to the outcomes. The Periphyton enriched in $\delta^{15}N$ longitudinally from site 1 to site 3. The results are agreed with recently study (Bergfur, *et al.,*2009; Mercado-silva, *et al.,* 2009). The likely reason is that high levels of anthropogenically derived from the surrounding the nutrient originated from human settings and agriculture activities along the river (Duda et al, 2011). In this sense, $\delta^{15}N$ can be potentially as sensitive indicators of watershed nutrient impacts like reported by other studies (Udy *et al.,* 2006; Bergfur, *et al.,* 2009; Winemiller *et al.,* 2011).

The $\delta^{13}C$ of Ayu showed a parallel variation with Periphyton, which indicated that temporal variation of $\delta^{13}C$ in primary producers, was conserved in consumers higher up the food chains since it has been reported that modification of $\delta^{13}C$ during feeding process is within 0-1‰ (France, 1995). Wada et al. (1987) established a relationship between $\delta^{15}N$ value and trophic level, TL (trophic level)=($\delta^{15}N_{animal}$-$\delta^{15}N_{algae}$)/3.3+1, in an Antarctic ecosystem. Using the $\delta^{15}N$ enrichment factor of 3.3‰ per trophic level, the trophic position of Ayu in the food web of the regulated reach was estimated to be 1.5-

2.2(1.8±0.3), indicating that the Ayu fish is the second trophic position and derived their biomass mainly from Periphyton. The isotope results are consistent with previous stomach content analysis.

CONCLUSION

In this study, we investigated the spatial and temporal variations in stable carbon and nitrogen isotope of Periphyton and an endangered fish in a flow-reduced river reach. The results showed that the Periphyton $\delta^{13}C$ showed a temporal and spatial variation. Both $\delta^{13}C$ and $\delta^{15}N$ of the Periphyton enriched longitudinally. The endangered fish (Ayu) was varied with the Periphyton during the study reach and trophic position analysis suggest that it was mainly feed on the Periphyton. The results can provide some information for studying the food web in flow-reduced reach and help us guild to conserve the endangered fish species in the river ecosystem.

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