

**Distributional patterns of benthic invertebrates at some meso-habitats of sandy riverbed of a mountain stream in Japan**

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**ABSTRACT**

Quantitative samples of benthic invertebrates were collected by a core sampler from 4 meso-habitats of a sandy riverbed at relatively slow flowing area of a mountain stream (Takami-gawa Stream, Nara Prefecture). A stretch of ca. 75 m was chosen for the samples collection, where riffle center (RC), riffle edge (RE), center of side pool (SP) and edge of sand bar (EB) were selected for sampling. The first one is a mid-channel habitat, while the others are marginal habitats of the channel. Sampling was carried-out over 5 sampling dates during the years 2008-2009. A total of 19967 individuals of benthic invertebrates, representing 120 taxa were identified. Comparisons using Two-way ANOVA test indicated that total abundance shows significant differences between habitats and seasons, with higher density at RC and in February 2009. Taxon richness exhibited only significant differences between seasons, with also higher value during February 2009. As well, Diversity index ( $H'$ ) showed significant differences between habitats and seasons, with lower values at RC and during May 2008. Dominant taxa such as *Paraleptophlebia*, *Potamanthus*, and *Zaitzevia* showed significant differences between habitats with higher abundance at RC. On the other hand, Oligochaeta exhibited higher abundance at RE. Benthic invertebrates were categorized into 4 groups according to their micro-vertical habitat; epifauna, fugitive fauna, occasional and permanent hyporheos. Occasional hyporheos accounted more than 50% in almost every habitat and sampling month, which was quite different from stony riffles assemblages. The insect larvae of *Paraleptophlebia*, *Potamanthus*, and *Zaitzevia* were predominant occasional hyporheos. This study confirms the suitability of core sampler to collect not only epifauna but also hyporheos in sandy riverbed.

**Keywords:** Benthic fauna, Epifauna, River, Sandy habitats, Bottom sampler, Hyporheos.

**INTRODUCTION**

The riffle-pool units of the streams have mosaic of meso-scale habitat, within some length of stream longitudinally, cross ward and vertically. These fine scale structural complexity enhances the diversity of benthic invertebrates.

Most studies on the Japanese streams have been conducted on habitats with faster current and/or stony stream beds, such as rapids or riffles. Using a surber net, Takemon and Tanida (1993) distinguished 11 types of meso-scale habitats within a reach of Takami-gawa Stream, which support variable riverine fauna, containing more than one hundred taxa. Takemon (1997) also reviewed the relationships of faunal composition to the structures of riffle-pools, bars, and hyporheic zones in the same river. He reported the importance of hyporheic zones in supporting younger nymphs of mayflies, which may occur widely in this zone of riverbed.

Graça *et al.* (2004) reported high content of organic matter and taxa richness in the marginal areas of 12 stream sites in Portugal. However, the fauna of marginal habitats in Japanese Streams were only partly revealed (Takemon and Tanida, 1993; Takemon, 1997).

The use of core sampler as a sampling device, which penetrates the substrata, coupled with fine-meshed sieve (125- $\mu$ m) could collect smaller-sized invertebrates of the hyporheic zone (Abdelsalam, 2012). This gives the chance to study the micro-vertical distribution of benthic invertebrates. This kind of distribution is always overwhelmed by other studies that use surface sampler such as surber net (Takemon and Tanida, 1993; Takemon, 1997).

Using a core sampler, the present work aimed to reveal the distributional patterns of benthic invertebrates within four target meso-habitats of sandy riverbed with a relatively slow flowing regime. The principal objectives of the present study are to compare the taxa richness, biological diversity and abundance of macro- to meio-benthic invertebrates in these meso-scale habitats, as well as the abundance of the dominant invertebrate taxa. An attempt has been done to expose the effect of water disturbance on the distribution of benthic invertebrate communities. Benthic invertebrates were classified into epifauna, fugitive, occasional and permanent hyporheic fauna, according to their micro-vertical distribution within riverbeds, in order to reveal the difference of composition of these faunal elements between meso-habitats and seasons.

## MATERIAL AND METHODS

### Study area

Kozu Site of Takami-gawa Stream was selected for the present study. Takami Stream is a tributary of Yoshino-gawa River in Nara Prefecture, central Japan. Takami-gawa itself is about 21 km long, which flows into the main channel of the Kino-kawa River (Taira and Tanida, 2011) (Fig.1). Kozu Site is a mountainous stream (34°23'17"N; 135°59'27" E; about 230 m above sea level) of the fourth order with a channel width of about 8- to 12-m and a mean slope of 0.009. The watersheds of this tributary are extensively forested with Japanese Cedar *Cryptomeria japonica* Done, 1894, whereas the riparian zones of the stream are well-covered with secondary deciduous vegetation (Taira and Tanida, 2011). Water does not freeze at the site over winter. Precipitation occurs mainly in summer season, especially from June through September.

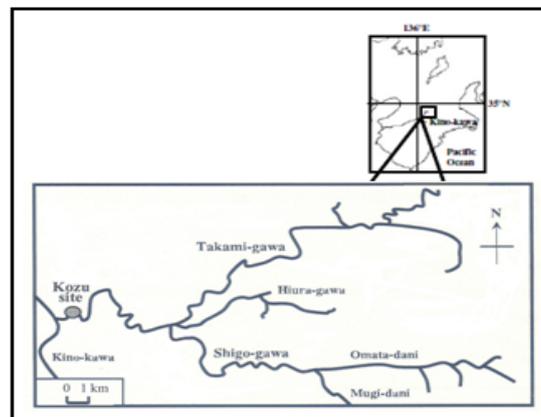


Fig. 1: Kozu Site, Takami-gawa Stream in Nara Prefecture, central Honshu, Japan. modified after Taira and Tanida (2011)

## Methods

Quantitative samples of benthic invertebrates were collected using a core sampler of 125 mm diameter (Tanida *et al.*, 2003) from sandy riverbed with a relatively slow flowing regime. Sampling was performed over 5 sampling dates through the years 2008-2009 namely: 26-27 April 2008, 20-21 May 2008, 21 August 2008, 7 November 2008 and 22 February 2009.

It is well-known that core sampler is suitable to reveal the vertical distribution of hyporheic benthos (Williams and Hynes, 1974; Godbout and Hynes, 1982). In the present study, the cores were inserted into the substrata for about 60-to120-mm depth, depending on the riverbed sediment composition.

A stretch of ca. 75 m including a riffle and a glide was selected for the collection of the samples. Four downstream target meso-habitats were investigated (Fig.2) at the site as follows: Riffle center (RC), Riffle edge (RE), Center of side pool (SP) and Edge of sand bar (EB). The first one is mid-channel habitat while others are marginal ones. Environmental conditions of each habitat such as current speed (by AEM1-D, Alec Electronics co. Ltd., Kobe, Japan) and depth were measured during sampling periods of November 2008 and February 2009.

In April 2008, only RC, RE and SP were sampled; but in the other 4 sampling months, all of the 4 habitats were surveyed. Three cores were collected from each habitat. Thus, a total of 57 benthic core samples were collected in the present investigation.

All samplings were performed under normal water level, however the river flow data near the site (the tentative report of water gauge station at Atarashi Site of Ministry of Land, Infrastructure and Transportation, Japanese Government) indicated that, the monthly average of water level based on hourly and daily data showed two peaks during June and September 2008 (Fig. 3a). In June 2008, the daily average of water level based on hourly data varied from 1.23 to 1.86m with two peaks through days of 3-4 and 22-24 June (Fig. 3b). Meanwhile, in September 2008, the daily average of water level ranged between 1.23 and 2.53m with sharp peak during days of 19-20 September (Fig. 3c).

Benthic invertebrate fauna and organic materials were separated from the substratum by elutriation using stream water. After washing sampled substratum several times in a bucket, the water was sieved through a 125- $\mu$ m net and the remaining material on the sieve was fixed in 5-10 % formalin solution. In the laboratory, all individuals were sorted and counted using a stereo microscope (Leica MS5, 6.3-40 magnification). Smaller specimens were mounted on slides and examined under a light microscope (Leica DME, 100-400 magnification). Most animals were identified to the lowest possible taxonomic level. Identification of specimens was carried out using relevant literature including: Merritt *et al.* (2008) and Kawai and Tanida (2005) for aquatic insects; Wiederholm (1983) for Chironomidae; and Uéno (1986) and Thorp and Covich (2001) for non-insect invertebrates. In the present study, the term taxon richness is used instead of species richness (Malmquist *et al.*, 2000), because some identifications were restricted to taxonomic level higher than genus. Most identification was made to the level of genus. However, Nematoda, Oligochaeta, Acari, Collembola and Tanypodinae are exceptions.

In all sampling months, one additional core sample was collected from each habitat (19 samples in total) for the grain size analysis. In the laboratory, organic matter in collected samples was excluded by repeated elutriation by water. After air drying for more than 7 days, inorganic particles were further dried at 70°C for 24 h in a drying oven (WFO-600ND, Eyela, Tokyo, Japan).

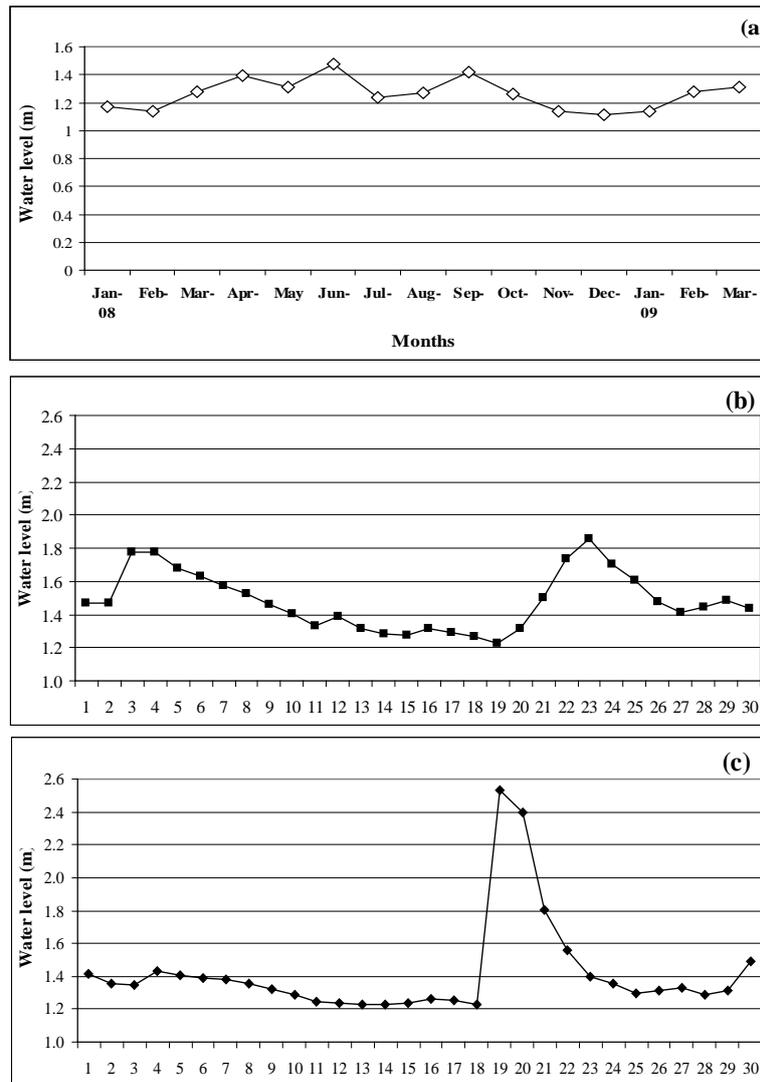


Fig. 3: Data of water level near Kozu site: (a) monthly average through the years 2008-2009, (b) daily average of water level during June 2008, and (c) daily average of water level during September 2008.

A series of sieves separated these inorganic particles into 8 size classes on  $\text{Log}_2$  scales: from 0.125 mm to 32.0 mm and each class was weighed to the nearest 10 mg by a mechanical balance. The weight percentages of these 8 size classes were calculated. The cumulative values of these 8 classes were plotted against  $\text{Log}_2$  scale of grain size. The median diameter ( $d_{50}$ ) of particle size for each sediment sample was calculated. The heterogeneity was determined using the cumulative values of sediment weight according to Schwoerbel (1961)

$$\text{Heterogeneity} = \frac{w_{60}}{w_{10}}$$

where  $w_{60}$  and  $w_{10}$  are the 60 and 10% cumulative weight percentage of inorganic sediments in each sample, respectively.

Particulate Organic Matter (POM) categories were measured for each core sample. Coarse particulate organic matter (CPOM, >1.0 mm) and fine particulate organic matter (FPOM, <1.0 mm) remaining after removal of invertebrates were dried at constant temperature (76°C) for 24 hr in the drying oven, weighed and ashed at 600°C for 2 hr (AT-E58, Isuzu Muffle Furnace, Niigata, Japan) to yield ash-free dry weight.

### Community parameters

The community parameters, *i.e.* taxa richness, total abundance as individual number/core area, and diversity index (Shannon-Wiener,  $H'$ ) of each habitat, were calculated. Some taxa such as Nematoda, Oligochaeta, Acari and Tanypodinae were identified only at higher taxonomic level (class to subfamily), which probably contained several genera and species. Therefore, these taxa were excluded for the calculation of taxa richness and diversity index.

### Classification of invertebrates by micro-vertical distribution

In the hyporheic zone, macro-invertebrates are usually present in significant numbers only for distances of 20 cm or less from the stream's channel (Williams and Hynes, 1974; Williams, 1984).

Benthic invertebrates were classified into 4 types: epifauna, fugitive fauna, occasional and permanent hyporheos. Fugitive fauna contain the taxa that exhibit a wide variety of life forms (epi- or hyporheic) and those without reliable information of vertical distribution. Williams (1984) suggested that the animals living in the interstices of streambeds could be divided into two types, occasional and permanent hyporheos. Occasional hyporheos consist of larvae (particularly of aquatic insects) of most of the surface benthos that may seek out this zone as a refuge during their early development. Permanent hyporheos, on the other hand, consists of many specialized groups that complete their life cycle there (*e.g.* Oligochaeta and Nematoda). For the classification into 4 types of fauna, some references of life forms and habitat traits were consulted; such as Merritt *et al.* (2008), Wiederholm (1983) and Thorp and Covich (2001).

### Data analyses

Differences between meso-habitats were analysed with the Scheirer-Ray-Hare extension of the Kruskal-Wallis test, as described in Sokal and Rohlf (1995). This is a non-parametric test analogous to Two-way ANOVA and allows comparing simultaneously (1) meso-habitats and (2) sampling dates or season. In this Two-way ANOVA test, we excluded the data of April 2008 because, only 3 meso-habitats (RC, RE and SP) were investigated. Three sets of comparisons were performed: (1) community parameters: taxa richness, diversity index and total invertebrate abundance/core unit, (2) masses of different POM categories (CPOM and FPOM) from each sampling unit, and (3) abundance of the dominant invertebrate taxa. Tukey post-hoc test was used to show the difference between meso-habitats or seasons if significant difference was detected.

The relationships between POM categories (CPOM and FPOM) masses from each sampling core were inspected against the taxa richness and abundance of benthic invertebrates, using Spearman rank correlation (Zar, 1996), and were conducted separately for each meso-habitat. These tests were performed using SPSS version 10 computer package.

## RESULTS

### Characteristics of the habitats:

Riffle center (RC) was the deepest sample (31-57 cm), with fastest current (mean water velocity  $\pm$  S.E. =  $0.450 \pm 0.05$  m/s). Riffle edge (RE) was shallow whereas the water depth fluctuated between (5 and 7 cm) with the slowest current ( $0.029 \pm 0.009$  m/s). Center of side pool (SP) was shallow (10-22 cm) and with low current speed ( $0.188 \pm 0.034$  m/s) habitat. Edge of sand bar (EB) was the shallowest (1.5-4.5 cm) with slow current ( $0.096 \pm 0.007$  m/s) (Table 1).

The highest median diameter of grain size was at RC and the lowest was at EB. The highest heterogeneity was also at RC and the lowest was at SP (Table 1).

Table 1. Current speed, water depth, sediment parameters and abundances of POM (particulate organic matter) at each habitat at Kozu Site, Takami-gawa Stream.

	RC	RE	SP	EB
Current speed (m/sec.)*	0.45 ± 0.039	0.029 ± 0.006	0.188 ± 0.024	0.096 ± 0.005
Mean ± SE				
Range	0.361- 0.579	0.012- 0.050	0.116 – 0.265	0.085 – 0.115
Water depth (range; m)*	0.31 – 0.57	0.05 – 0.07	0.10 – 0.22	0.015 – 0.045
Sediment parameters**				
Median diameter in mm (d50)	3.8 ± 1.29	2.2 ± 0.35	2.7 ± 0.80	2.1 ± 0.36
Mean ± SE				
Heterogeneity	7.1 ± 1.15	6.2 ± 1.5	4.4 ± 0.45	4.9 ± 1.31
Mean ± SE				
TPOM				
Mean ± SE (g/core)	0.0571 ± 0.0277	0.3446 ± 0.1022	0.5847 ± 0.3135	0.4684 ± 0.0998
Range	0.0035 – 0.4366	0.0818 – 1.6764	0.0571 – 4.8436	0.0750 – 1.2344
CPOM				
Mean ± SE (g/core)	0.0327 ± 0.0219	0.1624 ± 0.0654	0.3966 ± 0.2443	0.2166 ± 0.0613
Range	0.0008 – 0.3380	0.0066 – 1.0464	0.0124 – 3.7198	0.0451 – 0.6675
FPOM				
Mean ± SE (g/core)	0.0244 ± 0.0072	0.1821 ± 0.0393	0.1881 ± 0.0704	0.2518 ± 0.0478
Range	0.0027 – 0.0986	0.0414 – 0.6300	0.0291 – 1.1238	0.0299 – 0.5669

RC: riffle center, RE: riffle edge, SP: center of side pool, EB: edge of sand bar

\* measured only during November 2008 and February 2009 (6 readings for each habitat)

\*\* One sediment sample from each habitat was collected at each sampling occasion.

POM categories were measured for each core sample.

Amounts of deposited POM (CPOM and FPOM) were different between habitats and sampling months (Fig.4). Comparison of FPOM and CPOM between meso-habitats and seasons after the application of Scheirer-Ray-Hare extension of the Kruskal-Wallis test showed only significant differences for FPOM, between habitats ( $P = 0.0380$ , Table 2). Tukey post-hoc test indicated higher FPOM in EB than in RC. Results of Spearman rank correlation indicated highly significant positive correlation between FPOM and taxon richness and abundance of benthic invertebrates at RC habitat (0.750 and 0.632, respectively). On the other hand, taxon richness of SP habitat exhibited highly significant negative correlation (-0.623) and significant negative correlation (-0.534) with CPOM and FPOM, respectively (Table 3).

#### Composition of benthic invertebrates:

In total, 19967 individuals of benthic invertebrates, representing 120 taxa were identified throughout all sampling occasions. Of these, 4419 individuals were assigned to species, 11990 to genus, 1177 to subfamily, 240 to family, 251 to subclass, and 1890 to class. The taxonomic composition among different habitats and throughout all sampling occasions is presented in the Appendix. The benthic invertebrates of the investigated area were dominated by insects in terms of abundance and taxa.

The Ephemeropteran genus *Paraleptophlebia* dominated the benthic invertebrates in all habitats, constituting about 30% of the total abundance. The taxa

that individually formed more than 5% of the total abundance were *Paraleptophlebia* (Leptophlebiidae; Ephemeroptera) with relative abundance (ra): 29.6%, *Zaitzevia* (Elmidae; Coleoptera) (ra: 10.3%), *Oligochaeta* (ra:9.1%), *Potamanthus formosus* (Potamanthidae; Ephemeroptera) (ra:8.3%), *Nymphomyia alba* (Nymphomyiidae; Diptera) (ra:6.5%), Tanypodinae (Diptera) (ra:5.9%) and *Rheosmittia* (Chironomidae; Diptera) (ra:5.6%). These taxa constituted collectively about 75% of total benthic individuals.

Table 2: Comparison of community parameters and POM categories between seasons and microhabitats after the application of Scheirer-Ray-Hare extension of the Kruskal-Wallis test.

**Taxon richness**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Sig.
Season	789.563	3	263.1877	15.8860	0.0012	**
Microhabitat	26.729	3	8.9097	0.5378	0.9105	N.S.
Season* Microhabitat	692.354	9	76.9282	13.9302	0.1248	N.S.
within	827.333	32	25.8542			
total	2335.979	47	49.7017			

**Total abundance**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Sig.
Season	876372.729	3	292124.243	11.2517	0.0104	*
Microhabitat	1272919.396	3	424306.465	16.3429	0.0010	**
Season* Microhabitat	400488.521	9	44498.725	5.1418	0.8218	N.S.
within	1110968.667	32	34717.771			
total	3660749.312	47	77888.283			

**Diversity index (H')**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Sig.
Season	1.57	3	0.523333333	10.08887066	0.017825	*
Microhabitat	1.743	3	0.581	11.20057424	0.010689	*
Season* Microh.	1.025	9	0.113888889	6.586683074	0.680063	N.S.
within	2.976	32	0.093			
total	7.314	47	0.155617021			

**FPOM**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Sig.
Season	0.105	3	0.035	2.498734177	0.4755198	N.S.
Microhabitat	0.354	3	0.118	8.424303797	0.0380101	*
Season* Microh.	0.552	9	0.061333333	13.13620253	0.1565394	N.S.
within	0.964	32	0.030125			
total	1.975	47	0.042021277			

**CPOM**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Sig.
Season	1.649	3	0.549666667	5.359078966	0.1473144	N.S.
Microhabitat	0.971	3	0.323666667	3.155649288	0.3682439	N.S.
Season* Microh.	3.153	9	0.350333333	10.24692297	0.3308678	N.S.
within	8.688	32	0.2715			
total	14.462	47	0.307702128			

\* = < 0.05

\*\* = < 0.01

N.S. = Not Significant

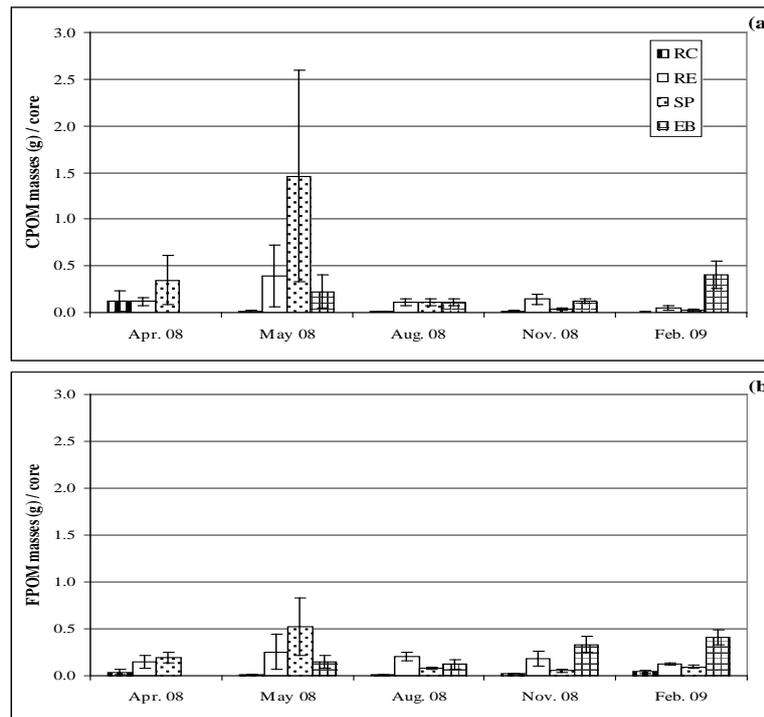


Fig. 4: Seasonal variations of different forms of POM (mean  $\pm$  SE); (a) CPOM and (b) FPOM, measured at different microhabitats of Kozu site of Takami-gawa River.

Table 3: Spearman correlations between different forms of POM and taxon richness, abundance of benthic invertebrate for samples of different meso-habitats of Kozu site.

		CPOM			FPOM		
		Correlation	Sig. (1-tailed)	Sig. (2-tailed)	Correlation	Sig. (1-tailed)	Sig. (2-tailed)
RC	Taxon richness	0.337	0.110	0.219	0.750**	0.001	0.001
	Abundance	-0.057	0.420	0.839	0.632**	0.006	0.011
RE	Taxon richness	-0.218	0.217	0.434	0.292	0.146	0.291
	Abundance	-0.450	0.046	0.092	-0.146	0.301	0.603
SP	Taxon richness	-0.623**	0.007	0.013	-0.534*	0.020	0.040
	Abundance	-0.339	0.108	0.216	-0.332	0.113	0.226
EB	Taxon richness	0.358	0.127	0.253	0.337	0.142	0.284
	Abundance	0.217	0.249	0.499	0.00	0.500	1.00

RC = riffle center, RE = riffle edge, SP = center of side pool and EB = edge of sand bar

\* Significant at the 0.05 level

\*\* Significant at the 0.01 level

### Community parameters:

Cumulative taxa richness varied among habitats and seasons (Fig. 5a), the maximum value (54 taxa) was in EB during Feb. 2009 while the minimum (13 taxa) recorded at Sp in April 2008. Cumulative taxa richness values of each habitat throughout the sampling months were 73 at RC, 83 at RE, 71 at SP and 78 at EB. Higher values were recorded in shallow retentive habitats (RE and EB).

Mean taxa richness was different between habitats and sampling months (Fig. 5b). As not all habitats were surveyed in April 2008, so if its data were excluded from the comparison, the highest value (33.3 taxa /core area) was in EB recorded during Feb. 2009 and the lowest (11.3 taxa /core area) was also in EB but during November 2008. Comparison of taxa richness between meso-habitats and seasons after the application of Scheirer-Ray-Hare extension of the Kruskal-Wallis test showed only

significant differences between seasons ( $P = 0.0012$ , Table 2). Tukey post-hoc test indicated higher taxa richness in Feb. 2009 than the other sampling dates.

Mean diversity index ( $H'$ ) was also different between habitats and sampling months (Fig. 5c). The maximum value (2.4) was recorded in EB during Feb. 2009 and the minimum (1.3) recorded in RC during May 2008. Comparison of diversity index indicated significant differences between meso-habitats ( $P = 0.0106$ ) and seasons ( $P = 0.0178$ ) (Table 2). Tukey post-hoc test for meso-habitats showed higher  $H'$  in SP and EB than in RC habitat. However, for the seasons, this test demonstrated higher  $H'$  in Aug. 2008 and Feb. 2009 than in May 2008.

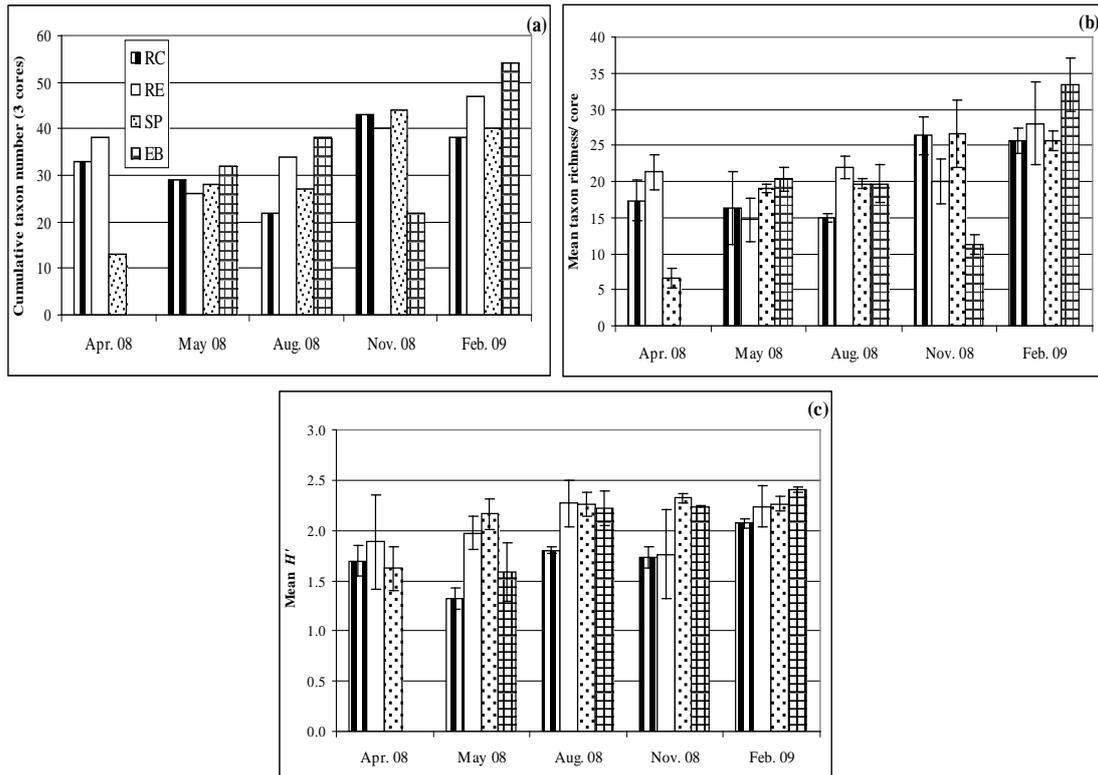


Fig. 5: Cumulative taxon richness, mean taxon richness and diversity index ( $H'$ ) at each habitat and sampling month of Kozu Site, Takami-gawa Stream.

The maximum total abundance of invertebrates (1041 individuals/core area) was recorded at RC habitat during Feb. 2009, meanwhile the minimum value (89 individuals/core area) was recorded at the EB during Nov. 2008 after excluding the data of April 2008 (Fig. 6a). Using two-ways ANOVA test, total abundance showed significant differences between meso-habitats ( $P = 0.0010$ ) and seasons ( $P = 0.0104$ ) (Table 2). Tukey post-hoc test for meso-habitats showed higher abundance in RC than the other habitats. However, for the seasons, this test demonstrated that Feb. 2009 > Aug. 2008 and Nov. 2008.

#### Comparison of abundance for dominant invertebrate taxa:

Application of two-way ANOVA test on abundance data for dominant invertebrate taxa revealed that some taxa such as *Paraleptophlebia*, *Zaitzevia*, *Oligochaeta*, and *Potamanthus formosus* only showed significant difference among meso-habitats ( $P = 0.004$ ,  $0.005$ ,  $0.009$  and  $0.000$ , respectively; Table 4). Tukey post-hoc test indicated that both *Paraleptophlebia* and *Potamanthus formosus* were more abundant in RC than the other meso-habitats. As well, *Zaitzevia* showed higher

abundance in RC than in SP. However, Oligochaeta showed higher abundance in RE than in SP.

Table 4. Comparison of the dominant taxa between seasons and microhabitats after the application of Scheirer-Ray-Hare extension of the Kruskal-Wallis test.

***Paraleptophlebia* (ra: 29.6%)**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Significance
Season	61066.562	3	20355.521	4.8111	0.1862	N.S.
Microhabitat	166034.062	3	55344.687	13.0809	0.0045	**
Season* Microhabitat	125413.854	9	13934.873	9.8807	0.3602	N.S.
within	244050	32	7626.563			
total	596564.479	47	12692.861			

***Zaitzevia* (ra: 10.3%)**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Significance
Season	324.063	3	108.021	0.4790	0.9235	N.S.
Microhabitat	8694.729	3	2898.243	12.8512	0.0050	**
Season* Microhabitat	6972.021	9	774.669	10.3049	0.3264	N.S.
within	15808	32	494.000			
total	31798.813	47	676.570			

***Oligochaeta* (ra: 9.1%)**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Significance
Season	1615.167	3	538.389	1.8987	0.5937	N.S.
Microhabitat	9744.167	3	3248.056	11.4549	0.0095	**
Season* Microhabitat	5068.667	9	563.185	5.9586	0.7441	N.S.
within	23552.667	32	736.021			
total	39980.667	47	850.652			

***Potamanthus formosus* (ra: 8.3%)**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Significance
Season	13778.25	3	4592.750	5.3014	0.1510	N.S.
Microhabitat	65410.083	3	21803.361	25.1676	0.0000	**
Season* Microhabitat	28000.25	9	3111.139	10.7736	0.2915	N.S.
within	14963.333	32	467.604			
total	122151.917	47	2598.977			

***Nymphomyia alba* (ra: 6.5%)**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Significance
Season	101352.167	3	33784.056	13.8036	0.0032	**
Microhabitat	53243.5	3	17747.833	7.2514	0.0643	N.S.
Season* Microhabitat	152622.333	9	16958.037	20.7862	0.0136	*
within	37878	32	1183.688			
total	345096	47	7342.468			

**Tanypodinae (ra: 5.9%)**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Significance
Season	7722.896	3	2574.299	17.9976	0.0004	**
Microhabitat	1617.396	3	539.132	3.7692	0.2875	N.S.
Season* Microhabitat	2595.688	9	288.410	6.0491	0.7350	N.S.
within	8232	32	257.250			
total	20167.979	47	429.106			

***Rheosmittia* (ra: 5.6%)**

Source of variation	SS	df	MS	SS/MS <sub>Total</sub>	P-value	Significance
Season	45437.167	3	15145.722	17.5327	0.0005	**
Microhabitat	14020.5	3	4673.500	5.4100	0.1441	N.S.
Season* Microhabitat	37468	9	4163.111	14.4577	0.1070	N.S.
within	24878	32	777.438			
total	121803.667	47	2591.567			

ra = relative abundance

Other taxa such as *Nymphomyia alba*, Tanypodinae, and *Rheosmittia* only exhibited significant differences between seasons ( $P = 0.003$ ,  $0.000$  and  $0.000$ , respectively; Table 4). Tukey post-hoc test showed that both *Nymphomyia alba* and *Rheosmittia* were more abundant in Feb. 2009 than the other sampling occasions. Tanypodinae also showed higher abundance in Feb. 2009 than in Nov. and Aug. 2008.

**Micro-vertical distribution of benthic fauna:**

The major elements of epifauna were *Ecdyonurus bajkovae* (Heptageniidae; Ephemeroptera), *Baetis* (Baetidae; Ephemeroptera), *Kamimuria* (Perlidae; Plecoptera), *Micropsectra* (Chironomidae; Diptera) and *Setodes* sp.1 (Leptoceridae; Trichoptera). Those of fugitive fauna were Tanypodinae, *Corynoneura* (Chironomidae; Diptera), *Orthocladius* (Chironomidae), Acari and *Caenis* (Caenidae; Ephemeroptera). The occasional hyporheos included *Paraleptophlebia*, *Zaitzevia*, *Potamanthus formosus*, *Nymphomyia alba* *Rheosmittia* and other taxa. This group contained two types of insect larvae and adults. The first type spends almost all its life cycle in hyporheic zone and appears at the surface of bottom only at pupation, emergence and reproduction, such as elmid beetles of *Zaitzevia*, *Ordobrevia* (Sato and Yoshitomi, 2005) and some stonefly nymphs of *Kiotina* and *Gibosia* (Shimizu *et al.*, 2005). Taxa of the second type spend their early stages as hyporheos, such as nymphs of *Paraleptophlebia* and *Potamanthus formosus*. Permanent hyporheos included members of only 2 groups, Nematoda and Oligochaeta.

In general, the maximum total abundance of invertebrates (590 individuals/core area) was recorded at RC habitat meanwhile; the minimum value (213 individuals/core area) was recorded at SP (Table 5). A total means of 104 individuals of epifauna belonging to 63 taxa formed of 25, 21, 33, and 25 individuals/core area were collected from RC, RE, SP, and EB respectively (The appendix; Table 5). As well, 198 individuals affiliating to 16 taxa of fugitive fauna were collected. A total of 948 individuals belonging to 39 taxa of occasional hyporheic and 135 individuals affiliating to only 2 taxa of permanent hyporheic were recorded. Hyporheic fauna were the most abundant in number (Fig. 6b). Occasional hyporheos were accounted for more than 50 % in every habitat and sampling month except in a few occasions.

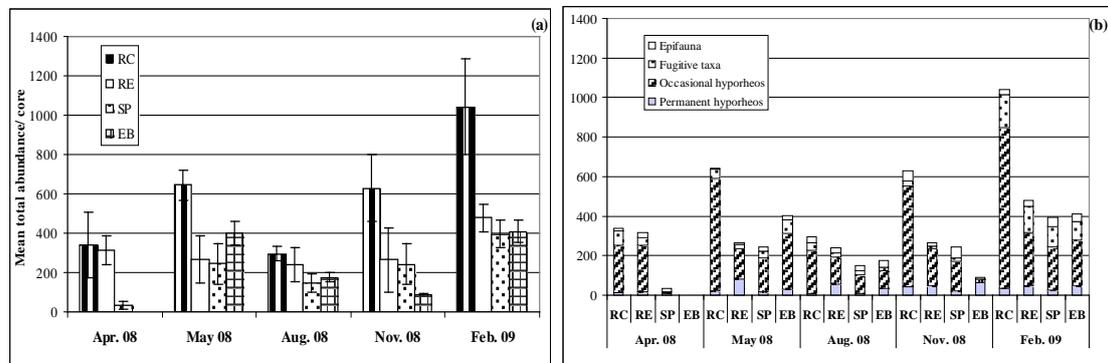


Fig. 6: Mean total abundance (a) and the faunal composition according to micro-vertical distribution in different habitats (b). Epifauna: surface dwelling animals, fugitive fauna: variable between species within the taxon (genus) treated or taxa without enough information on micro-vertical distribution, occasional hyporheos: spend a part of life in hyporheic zone, permanent hyporheos: spend the whole life in hyporheic zone.

Occasional hyporheos were less abundant in April (479 individuals/core area) than in other sampling months and they were the most abundant in February (1531 individuals/core area) (Table 5). Occasional and permanent hyporheos were less abundant at SP (127 and 17 individuals/core area) than the other habitats.

Table 5: Seasonal changes of abundance (number/core) of benthic invertebrates in different micro-vertical location at each habitat of Kozu Site, Takami-gawa Stream through the years 2008 – 2009.

		RC	RE	SP	EB	Total
Apr.08	Total abundance	340	315	35	n.d.	690
	Epifauna	14	24	17	n.d.	55
	Fugitive	75	38	6	n.d.	119
	Occasional hyporheos	241	236	2	n.d.	479
	Permanent hyporheos	11	17	10	n.d.	38
May 08	Total abundance	644	266	246	401	1557
	Epifauna	7	7	23	18	55
	Fugitive	48	24	33	70	175
	Occasional hyporheos	568	155	171	283	1177
	Permanent hyporheos	22	80	19	30	151
Aug. 08	Total abundance	296	240	148	176	860
	Epifauna	30	27	24	36	117
	Fugitive	39	22	22	16	99
	Occasional hyporheos	218	135	91	90	534
	Permanent hyporheos	10	56	10	34	110
Nov. 08	Total abundance	629	264	242	89	1224
	Epifauna	49	17	52	6	124
	Fugitive	29	12	19	1	61
	Occasional hyporheos	508	189	151	16	864
	Permanent hyporheos	43	46	20	66	175
Feb. 09	Total abundance	1041	479	394	410	2324
	Epifauna	27	31	49	38	145
	Fugitive	168	131	100	95	494
	Occasional hyporheos	813	271	219	228	1531
	Permanent hyporheos	34	47	27	48	156
Average total abundance		590	313	213	269	1385
	Epifauna	25	21	33	25	104
	Fugitive	71	45	36	45	198
	Occasional hyporheos	470	197	127	154	948
	Permanent hyporheos	24	49	17	45	135

RC: riffle center, RE: riffle edge, SP: center of side pool, EB: edge of sand bar

n.d.: Samples were not conducted at EB in April.

Oligochaeta was the most dominant permanent hyporheos throughout the sampling months (Table 6). Major taxa of occasional hyporheic were *Paraleptophlebia* and *Zaitzevia*, most of which were young instars just after hatching (personal observations). In February, *Nymphomyia alba* and a small-sized chironomid genus, *Rheosmittia* became abundant. Wide variety of fauna appeared as major epifauna taxa where *Ecdyonurus bajkovae*, *Baetis*, *Drunella basalis* (Ephemeroptera) and *Larcaria akagiae* (Goeridae; Trichoptera) were predominant.

Table 6: Dominant taxa of epifauna, fugitive, occasional hyporheos and permanent hyporheos recorded at each habitats of Kozu Site, Takami-gawa Stream.

	Habitat	Epifauna	Fugitive	Occasional hyporheos	Permanent hyporheos
<b>Apr-08</b>	<b>RC</b>	<i>Drunella basalis</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Larcasia akagiae</i>	Acari	<i>Zaitzevia</i>	
	<b>RE</b>	<i>Drunella basalis</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Synorthocladius</i>	Acari	<i>Zaitzevia</i>	Nematoda
	<b>SP</b>	<i>Semisulcospira</i>	Tanypodinae	<i>Potamanthus formosus</i>	Oligochaeta
		<i>Micronecta</i>	<i>Cryptochironomus</i>		Nematoda
<b>May-08</b>	<b>RC</b>	<i>Drunella basalis</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Larcasia akagiae</i>	Acari	<i>Potamanthus formosus</i>	Nematoda
	<b>RE</b>	<i>Baetis thermicus</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Tanytarsus</i>	Acari	<i>Zaitzevia</i>	Nematoda
	<b>SP</b>	<i>Aphelocheirus vittatus</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Larcasia akagiae</i>	<i>Dugesia japonica</i>	<i>Potamanthus formosus</i>	Nematoda
	<b>EB</b>	<i>Ecdyonurus bajkovae</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Larcasia akagiae</i>	Acari	<i>Zaitzevia</i>	Nematoda
<b>Aug-08</b>	<b>RC</b>	<i>Ecdyonurus bajkovae</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Baetis</i>	Acari	<i>Zaitzevia</i>	
	<b>RE</b>	<i>Micropsectra</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Kamimuria</i>	<i>Dugesia japonica</i>	<i>Zaitzevia</i>	Nematoda
	<b>SP</b>	<i>Kamimuria</i>	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Setodes</i> sp.1	<i>Dugesia japonica</i>	<i>Zaitzevia</i>	Nematoda
	<b>EB</b>	<i>Kamimuria</i>	Tanypodinae	<i>Zaitzevia</i>	Oligochaeta
		<i>Micropsectra</i>	<i>Dugesia japonica</i>	<i>Ordobrevia</i>	Nematoda
<b>Nov-08</b>	<b>RC</b>	<i>Ecdyonurus bajkovae</i>	<i>Caenis</i>	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Drunella basalis</i>	Tanypodinae	<i>Potamanthus formosus</i>	
	<b>RE</b>	<i>Setodes</i> sp.1	Tanypodinae	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Micronecta</i>	<i>Orthocladius</i>	<i>Zaitzevia</i>	Nematoda
	<b>SP</b>	<i>Ecdyonurus bajkovae</i>	<i>Caenis</i>	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Baetis</i>	Acari	<i>Zaitzevia</i>	
	<b>EB</b>	<i>Semisulcospira</i>	Tanypodinae	Ceratopogonidae	Oligochaeta
		<i>Setodes</i> sp.2	<i>Corynoneura</i>	<i>Zaitzevia</i>	Nematoda
<b>Feb-09</b>	<b>RC</b>	<i>Baetis</i>	<i>Corynoneura</i>	<i>Nymphomyia alba</i>	Oligochaeta
		<i>Ecdyonurus bajkovae</i>	Tanypodinae	<i>Paraleptophlebia</i>	
	<b>RE</b>	<i>Baetis</i>	<i>Orthocladius</i>	<i>Rheosmittia</i>	Oligochaeta
		<i>Stempellina</i>	<i>Corynoneura</i>	<i>Zaitzevia</i>	
	<b>SP</b>	<i>Baetis</i>	<i>Corynoneura</i>	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Ecdyonurus bajkovae</i>	Tanypodinae	<i>Nymphomyia alba</i>	
	<b>EB</b>	<i>Baetis</i>	<i>Corynoneura</i>	<i>Paraleptophlebia</i>	Oligochaeta
		<i>Ecdyonurus bajkovae</i>	Tanypodinae	<i>Zaitzevia</i>	Nematoda

RC: riffle center, RE: riffle edge, SP: center of side pool, EB: edge of sand bar

## DISCUSSION

Lotic habitats are composed of many microhabitats. This environmental mosaic affects the spatial distribution of the organisms (Scarsbrook and Townsend, 1993; Allan, 1995).

The present study mainly investigates the sandy riverbed in a mountain stream from mid-stream habitat (riffle center: RC) to margins of channels (riffle edge: ER, edge of sandbar: EB, and center of side pool: SP). No net samplers were adopted but core samplers with fine meshed sieve (0.125 mm mesh). These habitat characteristics

and sampling methods revealed different assemblages of stream benthic invertebrates from other studies conducted at the same stream (Kawanabe *et al.*, 1992; Takemon and Tanida, 1993; Takemon, 1997). Higher values of cumulative taxon richness were recorded in the shallow retentive habitats (RE and EB). It is well known that marginal microhabitat such as edge of sand bar is a source of great invertebrate diversity (Lindegaard, 1995). Principe and Corigliano (2006) reported that the marginal fauna was more diverse due to the asymmetry of transport and deposit processes, which generate a heterogeneous habitat in the bankside of river.

The present study indicated that *Paraleptophlebia* was the most dominant taxon within the 4 habitats and with higher density at RC. The result was in accordance with the finding of Holomuzki and Messier (1993), who indicated that the larvae of *Paraleptophlebia guttata* were significantly denser both in runs and riffles than pools in the second order stream of west-central, Kentucky, USA. They mentioned that considering the *Paraleptophlebia* as generally poor swimmers, the cost of using pools with less protective substrata outweighs the benefits of lower current velocity and higher food density. Higher abundances of *Zaitzevia*, and *Potamanthus formosus* were also recorded at RC. Many authors (*e.g.* Hynes, 1970; Logan and Brooker, 1983) have found that riffles, in general, always include a greater number of organisms and total biomass. However, the majority of these studies dealt with upland streams with predominantly stony substrates. Oligochaeta showed higher abundance at RE habitat. However, it was the most dominant permanent hyporheos at the area of study. Oligochaetes high abundance can be related to the worm-shaped body that is well fitted to an interstitial existence and that dominated the sediment fauna of different types of rivers (*e.g.* Boulton and Foster, 1998; Gibert *et al.*, 1998).

Results of FPOM and diversity index ( $H'$ ) indicated higher values in EB than in RC. These results agree with Graça *et al.* (2004) who reported high abundance of organic matter and taxa richness in shallow retentive areas. It is worthy to mention that the marginal habitat of EB represent the shallow retentive area of the stream which harbours high taxon richness and FPOM. However, Canhoto and Graça (1998) found that physical traits, such as water velocity or the presence of shallow margins, enhance the leaf litter retention efficiency of the stream patches.

At the central habitat (RC), FPOM mass showed positive correlation with taxon richness and abundance of benthic invertebrates, while in SP, taxon richness showed negative correlations with both CPOM and FPOM. It is well known that, CPOM accumulations are the main source of FPOM which could benefit invertebrate assemblages. González and Graça (2005) also reported positive correlation between CPOM mass and total density in riffles and sandy pools of a fourth-order reach of a small Portuguese stream. This relationship could be interpreted as a consequence of detritus increasing the structural complexity of the habitat (Downes *et al.*, 1998; Stewart *et al.*, 2003). On the other hand, the negative correlations between taxon richness and POM categories estimated at SP habitat, suggested that the influence of POM categories on taxon richness was due to increasing their density or decreasing their equality in distribution. However, it is possible that there are other unstudied factors that may explain this result.

During November 2008, the least mean taxon richness (11.3 /core area) and total abundance (89 individuals/core area) were recorded at EB habitat. From the river flow data near the site (the tentative report of water gauge station at Atarashi Site of Ministry of Land, Infrastructure and Transportation, Japanese Government), two days of flood were recorded in the 19<sup>th</sup> and 20<sup>th</sup> of September 2008, which was the flood of about one year recurrent interval. The maximum water level (4.07m) was about 3

times higher than the normal level. The conditions imposed by the extremes of such temporal variability are often referred to as disturbance. Disturbance is a fundamental determinant of the structure of stream communities (Lake, 2000). Floods can cause significant mortality of aquatic organisms (Fisher *et al.*, 1982). No benthic samples were collected just after the flood. But the influence of the flood at EB habitat during November could be detected.

Although samples of November 2008 were collected more than one month after the high water at Yoshino-gawa Stream, it is likely that the flood might cause the low taxon richness and abundance at the marginal habitat (EB). Resh *et al.* (1988) have indicated that all ecological phenomena in lotic ecosystems are temporally affected to some extent by extremely high or low water flows. In the present study, it seems that EB was more affected by the flood disturbance than other marginal habitats. Owing to the inwardly spiralling flow patterns in riffles (Richards, 1982). It is presumed that the centers of riffles (in cross section) receive fewer disturbances.

Regarding the seasonal variations, the higher taxon richness, diversity index ( $H'$ ) and abundance of invertebrate communities during February 2009 might be related mainly to the life history traits of most aquatic insect taxa, whose larvae and nymphs emerge during spring season. Populations of different species of stream insects often show their highest and lowest individual growth rates during different periods of the annual cycle (Vannote and Sweeney, 1980). Warm temperatures are often correlated with rapid rates of insect growth and development. Strategies enabling such differences in growth patterns often involve diapauses or extended periods of quiescence during high summer or low winter temperatures, and rapid growth during optimal thermal conditions (Huryn *et al.*, 2008).

In the studies of riffle or fast flowing areas of stream with rocky substrata, and by using surface net sampler, the epifauna is the major constituent of benthic invertebrates. However, in the present study, the occasional and permanent hyporheic fauna formed the dominant invertebrates of the sandy habitats. It has been revealed that the sampling devices using cores may enhance the efficiency of collecting hyporheic fauna from sandy riverbed (Williams and Hynes, 1974; Godbout and Hynes, 1982; Gillespie *et al.*, 1985; Tanida *et al.*, 2003). In classification of the 4 life forms according to micro-vertical distribution or location, occasional hyporheos were predominant throughout all habitat and sampling months. Among occasional hyporheos, *Paraleptophlebia* and *Potamanthus formosus* were major fauna. These aquatic insects spend only early stages of nymphs in hyporheic zones and later instar nymphs stay as epifauna. The hyporheic zone is considered an important refuge for several surface invertebrates (Hose *et al.*, 2005). In contrast to those mayfly nymphs, the larvae of some elmids and stonefly nymphs spend almost all larval stages as hyporheos and appear on the surface of stream bottom for emergence, pupation and oviposition (Shimizu *et al.*, 2005).

The high abundance of occasional hyporheos collected during May and November 2008 is a result of accumulation of the very small stages of *Paraleptophlebia* and *Potamanthus formosus* which temporarily inhabit the interstitial space just after hatching (personal observations). Williams (1984) indicated that the early instars of many species of benthic stream insects occurred deep within the stream bed if suitable interstices were present. These include elmids, caddisfly larvae (*Cheumatopsyche*, *Helicopsyche borealis*), small nymphs of mayfly genera (*Caenis*, *Ephemerella* and *Paraleptophlebia*), young nymphs of the plecopteran species *Allocapnia pygmaea*, and chironomid larvae (*Cricotopus*, *Cladotanytarsus*, *Microtendipes* and *Orthocladius*). In the present study,

a part of these fauna, *Caenis*, *Paraleptophlebia* and some chironomid genera were also collected as occasional hyporheos or fugitive fauna. Oligochaeta was the most dominant permanent hyporheos throughout the sampling months. Mary and Marmonier (2000) also reported the dominance of oligochaetes in the hyporheic communities of the New Caledonian Rivers.

This study highlights the efficiency of core sampler coupled with fine-meshed sieve (125- $\mu$ m) for collecting small hyporheic fauna from 4 different micro-habitats of a sandy riverbed. RC (central habitat) showed higher abundance of benthic community than the other marginal habitats. On the other hand, higher taxon richness was recorded in the marginal retentive habitats (RE and EB) indicating the importance of these habitats for conserving the biodiversity. In every surveyed habitat, the occasional and permanent hyporheic fauna formed the major constituent of benthic invertebrate, which have been often overlooked by net samplings of stream benthic invertebrates, because of their micro-habitat and small body size. The present work also demonstrates that edge of sand bar is more affected by water disturbance than the other marginal habitats.

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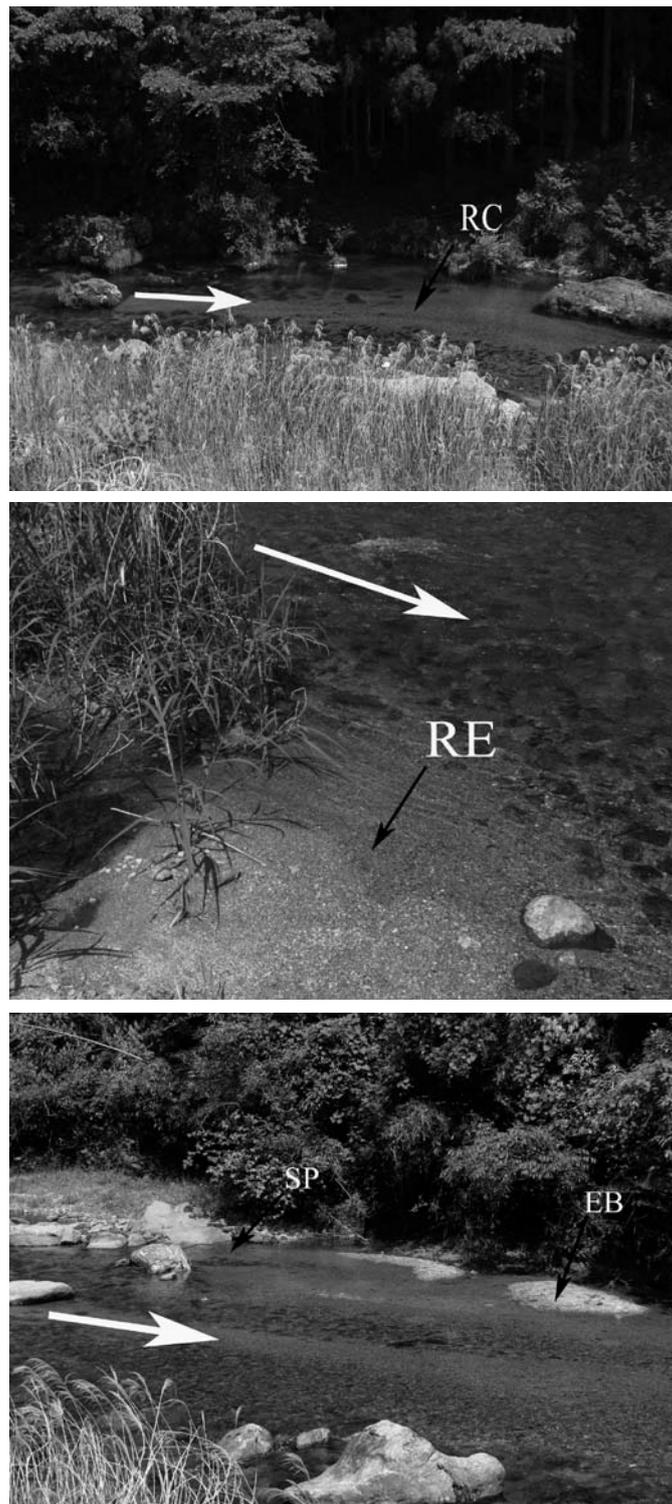


Fig. 2: Four meso-habitats were investigated at the Kozu Site, Takami-gawa Stream. RC: riffle center, RE: riffle edge, SP: center of side pool, EB: edge of sand bar. White arrows show flow direction.

## ARABIC SUMMARY

## أنماط توزيع اللافقاريات القاعية في بعض الموائل الوسيطة لقاع نهر رملي جبلي في اليابان

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تم تجميع عينات كمية من اللافقاريات القاعية بواسطة جامع عينات القاع الأسطواني من أربع بيئات وسيطة لقاع نهر رملي في منطقة تدفق بطيئة نسبياً لنهر جبلي (نهر تاكامي، ولاية نارا). وقد تم اختيار قطاع بطول ٧٥ متر لجمع العينات، حيث تم إنتقاء البيئات الأربع التالية لأخذ العينات: وسط منحدر النهر (RC)، حافة منحدر النهر (RE)، وسط بركة جانبية (SP)، وحافة شريط رملي (EB). وقد مثل الأول بيئة منتصف القناة، في حين أن الثلاثة الآخرين مثلوا البيئات الهامشية للقناة. وقد تم تحديد ما مجموعه ١٩٩٦٧ فرد من اللافقاريات القاعية التي تمثل ١٢٠ وحدة تصنيفية.

وقد تم تجميع العينات في خمسة أوقات علي مدار العامين ٢٠٠٨-٢٠٠٩. وباستخدام الإختبار الأحصائي (Two-way ANOVA) أظهر مجموع الوفرة فروقاً ذات دلالة إحصائية بين البيئات والمواسم المختلفة مع كثافة مرتفعة في بيئة RC وخلال شهر فبراير ٢٠٠٩. وقد أظهر ثراء الأصناف فروقاً ذات دلالة إحصائية فقط بين المواسم مع عدد أصناف أكبر خلال شهر فبراير ٢٠٠٩. وكذلك أظهر مؤشر التنوع الحيوي ( $H'$ ) فروقاً ذات دلالة إحصائية بين البيئات والمواسم مع قيم منخفضة في بيئة RC وخلال شهر مايو ٢٠٠٨. كما أظهرت الأنواع السائدة مثل *Paraleptophlebia*، *Potamanthus*، و *Zaitzevia* فروق ذات دلالة إحصائية بين البيئات مع كثافة مرتفعة في بيئة RC، ومن ناحية أخرى، أظهرت مجموعة *Oligochaeta* أعلى وفرة في بيئة RE.

وقد صُنفت اللافقاريات القاعية إلى أربع مجموعات وفقاً للتوزيع الراسي الدقيق للبيئات إلى حيوانات سطحية وحيوانات تضم مجموعة متنوعة من أشكال الحياة، حيوانات عرضية المعيشة في المنطقة الخلالية للرسوبيات، وحيوانات دائمة المعيشة في نفس المنطقة. وقد شكلت الحيوانات عرضية المعيشة في المنطقة الخلالية للرسوبيات تقريباً أكثر من ٥٠٪ من العدد الكلي في كل بيئة وفي كل شهر، والذي كان مختلفاً تماماً عن تجمعات منحدرات الأنهار الصخرية. وكانت يرقات الحشرات للأنواع *Paraleptophlebia*، *Potamanthus*، و *Zaitzevia* هي الحيوانات العرضية الغالبة. هذه الدراسة تؤكد ملاءمة جهاز جمع عينات القاع الأسطواني لجمع ليس فقط الحيوانات السطحية ولكن أيضاً الحيوانات التي تعيش في المنطقة الخلالية للرسوبيات قاع النهر الرملي.