

# Longitudinal patterns of benthic invertebrates along a stream in the temperate forest in Japan: in relation to humans and tributaries

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**Abstract.** 1. The relationship between benthic invertebrate assemblage composition and surrounding land use has been examined in various ways, but most studies are undertaken at a coarse scale, or they compare obviously different landscapes. In Japan, these obvious differences in landscape are rare, and humans reside even in remote montane areas.

2. In order to clarify the influence of human residence on benthic invertebrate assemblages, seasonal and site differences among benthic samples collected from a 15-km stretch of Kuroson Stream and their relation with riparian land use were examined.

3. The number of individuals and genera differed significantly both spatially and temporally. Multivariate assemblage structure also differed significantly across space and time along the stream.

4. Increase of residential areas affected the in-stream benthic invertebrate assemblage. Site differences along the stream and the effect of human residence were not masked by the potentially homogenizing influence of tributary streams.

5. The composition of surrounding vegetation was the most important factor influencing benthic community structure. The presence or absence of human residential areas and seasonal change were also important variables.

6. Benthic invertebrates may be carried and migrate to main streams from tributaries; however, this phenomenon was not observed. Assemblages of benthic invertebrates that inhabit a particular site do not change and are considered to vary seasonally across a certain range.

**Key words.** Benthic invertebrates, drift, forest type, human residence, land use, riparian.

## Introduction

Benthic assemblages in streams and rivers are affected by physico-chemical factors such as temperature, channel stability, seasonal shifts in weather, pathways of water, and the frequency of periods of favourable environmental conditions (Burgherr & Ward, 2001). Preferred water temperature differs and is dependent on the classification just like the preference of lower water temperature for stonefly and mayflies (Brittain, 1990). Substrate heterogeneity is created by disturbance and variability in physical conditions (Reice, 1994). This heterogeneity produces patchiness in environmental conditions, including food availability, which in turn results in high species diversity in benthic invertebrates (Godbout & Hynes, 1982).

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Benthic assemblages are also affected by riparian condition. Riparian land use varies with stream location; some riparian areas are fully vegetated, while others have been anthropogenically converted to home sites, paddy fields or farms. Riparian land use changes the stream habitat structure (including nutrient inputs) with consequent effects on aquatic invertebrates (Krueger & Waters, 1983; Sweeney, 1992; Allan *et al.*, 1997). The development of riparian vegetation plays an important role in the succession of benthic invertebrates (Flory & Milner, 1999), and the quality of nutrients in the stream derived from riparian forests affects larval growth (Fiance, 1978; Malley, 1980; Hawkins *et al.*, 1982; Rodgers, 1984). When riparian land is covered with forests and/or wetlands, biotic integrity and habitat quality in the stream increase, but when riparian land is used for agriculture these measures of environmental health decline (Roth *et al.*, 1996). Water quality in forest streams is

different from that of urban streams that have a lower abundance of aquatic invertebrates (Hall *et al.*, 2001). Recognition of the variability of riparian and in-stream landscapes is necessary to obtain a better understanding of the life cycles of benthic invertebrates (Reed *et al.*, 1994; Richards *et al.*, 1996).

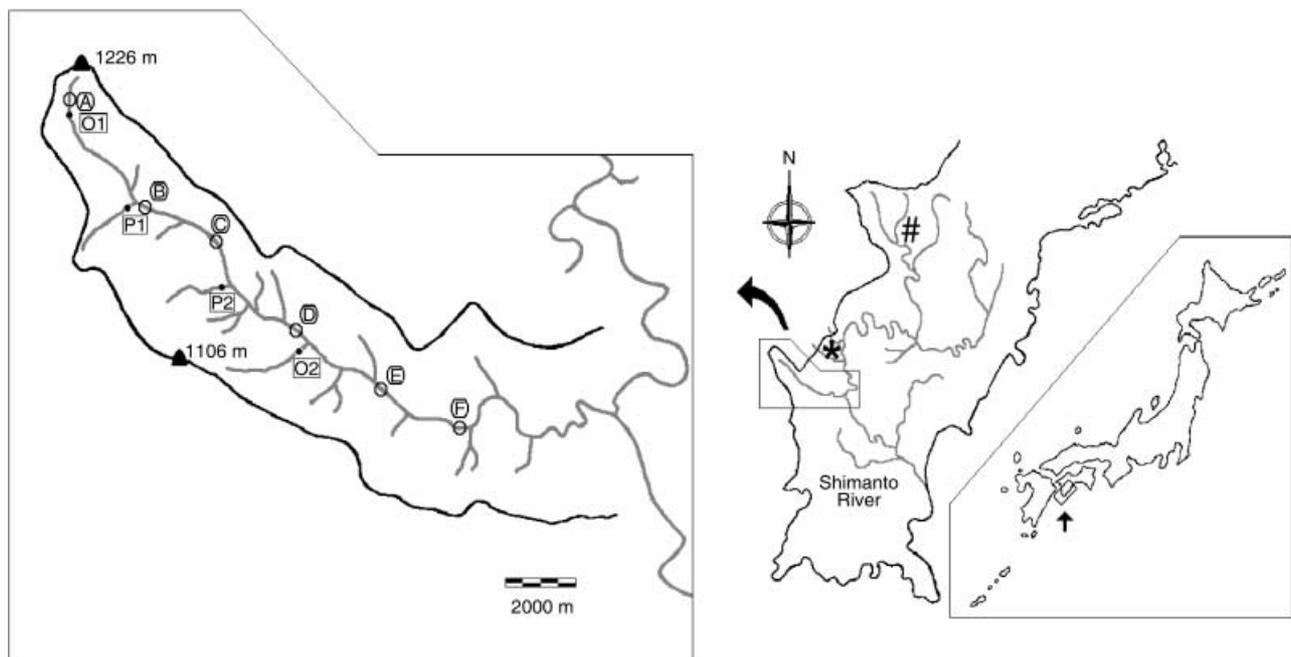
Various components of the relationship between surrounding land use and benthic invertebrate assemblages have been studied, but most have been examined at large scales or compared among obviously different landscapes. In remote areas of Japan, there is no obvious change of land use along water courses (e.g. from forest to pasture and from forest to urban). Land management is patchy and rarely carried out at large scales. Furthermore, there are human residences in even the most remote forested mountain areas. This status is peculiar to Japan in the world, where forested mountain area is more than 65% and population is more than one hundred million. Urban human communities invariably impact water quality and the composition of invertebrate benthos, and we suspect that there are effects in even remote regions of Japan. However, differences in benthic invertebrate assemblages along streams in these remote forested regions have not been well studied in Japan. In order to examine the impact of human communities on benthic invertebrates, spatial and seasonal differences of benthic invertebrate assemblages were examined along 15 km of a stream water course in a remote area. The relation between benthic invertebrate assemblage structure and riparian and in-stream conditions were analysed. Human effects on stream benthos may be masked by inflows from tributaries. When the invertebrates in tributaries drift down to the main stream regularly, benthos structure may be similar between tributaries and the main stream. Accordingly, we examined benthos in both the main and tributary streams in the temperate region of Japan.

## Materials and methods

### Study sites

The study area is in the Shimanto River watershed of Shikoku, the fourth largest island of the Japanese archipelago (Fig. 1). The watershed is covered by lowland and hill forests up to 1400 m above sea level (a.s.l.). The annual mean temperature and precipitation from 1995 to 2004 at Yusuhara (415 m a.s.l.; Fig. 1, #) and Ekawasaki (60 m a.s.l.; Fig. 1, \*), respectively, were 13.4°C and 2733 mm, and 15.6°C and 2375 mm. Vegetation was composed principally of conifer plantations (about 60% of the total forested area), secondary forest (about 40%), and remnants of old-growth forests (< 1%) with no historical record of having been cleared.

The old-growth forests were dominated by evergreen conifers (e.g. *Abies firma*, *Tsuga sieboldii*, and *Chamaecyparis obtusa*) and diverse evergreen broad-leaved trees (e.g. *Quercus* spp., *Castanopsis cuspidata*, *Machilis japonica*, and *Cleyera japonica*). At around 800 m a.s.l., evergreen broad-leaved trees were gradually replaced by deciduous trees (e.g. *Lindera erythrocarpa*, *Mallotus japonicus*, *Carpinus* spp., *Fagus* spp., *Betula grossa*, and *Acer* spp.). The lowland secondary broad-leaved forests consisted of coppices dominated by evergreen broad-leaved trees (e.g., *C. cuspidata* and *Quercus glauca*), whereas those at higher altitudes consisted of deciduous broad-leaved trees (e.g., *Quercus serrata* and *Carpinus* spp.). The conifer plantations consisted of monocultures of Japanese cedar (*Cryptomeria japonica*) or Japanese cypress (*Cryptomeria obtusa*), which were cut at 30- to 80-year intervals. Managed plantations were thinned, and their forest floors were covered by abundant shrub species.



**Fig. 1.** Study site in the Shimanto watershed, Kochi Prefecture, Japan. ○, sampling sites A–F; ●, sampling sites O1–O5 and P1–P5. #, Yusuhara; \*Ekawasaki.

Six sampling sites (A–F) were selected along Kuroson Stream (Site A: 33°10'N, 132°38'E; site F: 33°05'N, 132°45'E), a tributary of the Shimanto River (Fig. 1). Two sampling sites (O1, O2) were established in each of two natural old-growth forest watersheds; one watershed is in the upper reaches of the main stream, and the other drains as a tributary of Kuroson Stream into the upper reaches of site E (Fig. 1). Two sampling sites (P1, P2) were established in each of two planted coniferous forest watersheds of tributaries of Kuroson Stream (Fig. 1); one tributary flowed into the upper reaches of site B, and the other flowed into the upper reaches of site D. These two watersheds were vegetated with natural old-growth forest, which pre-dated cutting and afforestation that took place about a century before our study. The surface soil at these sites was categorised as Shimanto Terrain and consisted of alternating sandstone and mudstone beds.

#### *Sampling of benthic invertebrates and measurement of environmental factors*

Five replicate samples of benthic invertebrates were collected from each site along Kuroson Stream at 2-month intervals from August 2002 to June 2003. Five replicate samples of benthic invertebrates were also collected from each site in two natural old-growth forest watersheds and two planted coniferous forest watersheds in December 2000 or 2001. Weather was fine on all sampling days, and obvious changes of water level were not observed. A 30 × 30-cm quadrat was established on the substrate in each sampling site in shallows of 20–25 cm depth with a flow rate of 0.15 to 0.2 m/s. A D-frame net with 1-mm mesh was placed downstream of the quadrat. Large stones in the quadrat were removed and placed directly into a white tray. The substrate surface in the quadrat was then disturbed, allowing invertebrates to drift into the net. All invertebrates on the stones and in the net were removed and preserved in 80% ethanol. The preserved benthic invertebrates were later identified to genus using a 50× microscope (SMZ-U; Nikon, Japan), except for Chronomidae and Tipulidae, which were identified to subfamily.

Land use along Kuroson Stream was determined using a 1:25 000-scale map and field surveys. Buffer zones of 250 m width on each side of the mainstream-line were divided into 125 × 125-m grids, and the number of grids covered by forested areas and residential including road and fields areas were counted. When two categories were found within one grid, each was assigned a score of 0.5 grid. The percentages of forested and residential areas at each site were calculated between the site and the nearest upper reaches site in the 250-m buffer zones on either side of the main stream.

At each site where benthic invertebrates were collected, water and air temperature, electrical conductivity (EC), pH and dissolved oxygen (DO) were measured using a portable thermometer (5473, TANITA, Japan), a compact twin conductivity metre (B-173; Horiba, Japan), a compact twin pH metre (B-212; Horiba) and a DO metre (DO-5509; Lutron, Korea), respectively. The width of the stream (SW) was also measured using a portable metre (Yardage Pro; Bushnell, U.S.A.).

Algal samples were collected at 2-month intervals from August 2002 to June 2003 on five stones randomly selected in

each site along Kuroson Stream. Collections and extraction of algal chlorophyll followed the methods of Tanida *et al.* (1999). Dried tissue (CK-009; ELECOM Co.) made of Shalera™ (ASAHI KASEI FIBERS Co.) was used as collecting material. A 5 × 5-cm quadrat was established on the stone surface in the stream, and the brown algae in the quadrat on each stone were scraped off with the dried tissue. Algae trapped on the dried tissues were soaked in 30 mL of ethanol (99%) for 24 h in the dark to extract chlorophyll pigments. The absorbance of the extracted chlorophyll pigments was measured at 750, 663, 645, and 630 nm using a spectrum luminous intensity metre (V-560; JASCO Co.). The density of chlorophyll *a* and chlorophyll *c* per unit area (m<sup>2</sup>) in each quadrat was calculated according to SCOR-UNESCO-1966 methods and averaged for each site.

#### *Statistical analysis*

The percentage cover of forested and residential areas, altitude and stream width at each site were assessed among six sites using the Ward method, with clustering by Euclidean distance. Water and air temperature, conductivity, pH and DO data collected every 2 months at each site were assessed for 36 samples using the Ward method, with clustering by Euclidean distance at which the samples could be divided into two groups. Friedman non-parametric two-way analysis of variance was used to compare environmental factors, i.e. water and air temperature, conductivity, pH, DO, and the density of chlorophyll *a* and chlorophyll *c*, between six sites along Kuroson Stream (i.e., A–F) between six sampling months (i.e., August–June).

Abundance data were pooled for each site, and the number of individuals, number of genera and families, and The Shannon–Wiener  $\alpha$ -diversity of sites were compared between six sites and 6 months using Friedman two-way analysis of variance. Correlations between the four diversity indices and the stand variables were tested using Spearman's rank correlation.

Correlation coefficients among 36 samples [six sites (A–F) for 6 months] of the benthic invertebrate assemblage were obtained using a Spearman's rank correlations test on the abundance data for each genus. These correlation coefficients were analysed using principal components analysis (PCA). Correlations between the PCA axes and several stand variables were examined using Spearman's correlation test. These correlation coefficients of genus abundance among the 36 samples along Kuroson Stream were also assessed using the Ward method and sites were clustered by Euclidean distance at which the cluster include less than four sites. To determine the genera that tended to occur in particular sites and/or months, Friedman two-way analysis of variance was used to compare the abundance among sites and months. This analysis was performed for genera in which at least five individuals were collected (based on the assumption that at least one individual per quadrat could be collected).

Correlation coefficients for the benthic invertebrate assemblages between 10 sites collected in December only (A–F in December, O1, O2, P1, and P2) were obtained employing Spearman's rank correlation on the abundance data for each

genus. The correlation coefficients were analysed using PCA. The correlation coefficients among 10 sites were also assessed using the Ward method and sites were clustered by Euclidean distance at which the cluster included less than four sites. Using a Kruskal–Wallis test, the value of axis 1 of PCA was compared between the groups categorised by cluster analysis. To determine the genera that tended to occur in either group, Kruskal–Wallis one-way analysis of variance was used. This analysis was performed when the genera had at least five individuals in all sites (based on the assumption that at least one individual per quadrat could be collected). All statistical analyses were performed using SYSTAT version 10 (SPSS Inc. 2000).

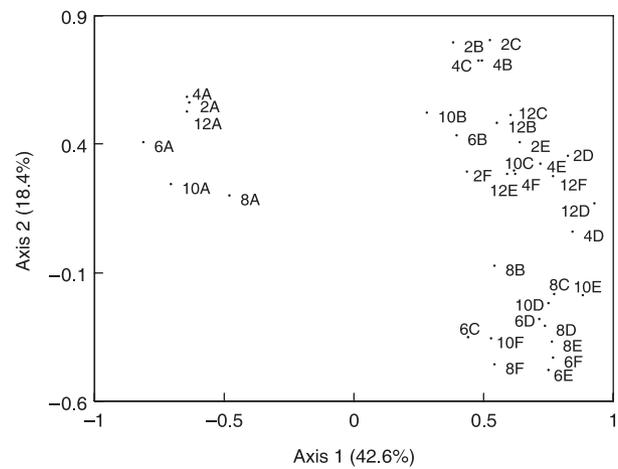
## Results

At site A, the width of the stream was 5 m and the altitude was 550 m, while at site F, the stream width and altitude were 26 m and 90 m, respectively. In addition to the downstream changes in stream width and altitude, the proportion of residential area increased, whereas that of forest decreased (Table 1). Forest at site A was old growth natural trees, and forest at the other sites comprised a mixture of broadleaved and coniferous trees. The percentage of forested and residential area, altitude and stream width were highly correlated [Spearman's rank correlation ( $\rho$ ): > 0.9], and these four factors at six sites were divided into two groups (A–C and D–F) by similarity analysis.

DO and air temperature differed significantly only among sampling months (Table 1). DO was highest in February and air temperature was highest in August. EC and water temperature differed among sites and months. The pH did not differ significantly among sampling months and sites along Kuroson Stream. Similarity analysis showed that data for these factors (water and air temperature, conductivity, pH and DO) collected at six sites every 2 months were firstly divided into two groups (December–April and June–October). The density of chlorophyll *a* and *c* varied significantly with time only (Table 1). The density of chlorophyll *a* was highest in October, and that of chlorophyll *c* was highest in December.

In total, 15 688 individuals from 60 families and 101 genera of benthic invertebrates were collected at the six sites throughout the year. The number of individuals, families, and genera differed significantly over time among six sites along Kuroson Stream (Table 2). Significant site differences in the number of individuals were detected between sites A and B, and in the number of genera between sites A and D. Significant differences between months were detected in four indices, with the lowest numbers in June or April (Table 2). The number of individuals was positively correlated with the density of chlorophyll *a* and chlorophyll *c*. The number of families and genera was positively correlated with pH. Diversity was not correlated with any factor (Table 3).

The first two axes of the PCA ordination of benthic invertebrate genus composition in the 36 samples from Kuroson Stream explained 61.0% of the variation (Fig. 2). Site A samples for all months were clustered to the left, whereas site B–F samples were clustered to the right of the ordination diagram (Fig. 2). Axis 1 showed significant differences among sites, whereas axis



**Fig. 2.** Principal components ordination diagram of benthic invertebrate genus composition. Letter, sampling site; number, sampling months.

2 showed significant differences among both sites and sampling months (Table 4). Axis 1 was significantly correlated with the percentage of forested and residential area, altitude and stream width. Axis 2 was correlated with almost all of the factors (Table 4).

Genus composition of benthic invertebrates in 36 samples from Kuroson Stream was divided into five groups when the Euclidean distance of 0.3 was selected (Fig. 3). First, they were divided into two groups (Site A and other sites). Second, the genus composition of the sites except site A were divided into two groups dependent on month, and third they were divided into four groups dependent on site (B–C and D–F) and month (December–April and June–October). Benthic invertebrate assemblages along the stream appeared to differ along stream according to the period of the year (December–April and June–October) and site (A, B–C and D–F).

Abundance at the six sites was significantly different in 32 genera and pairwise differences between sites were detected for 14 genera (Table 5). The number of individuals of *Togoperla* spp. and *Calineuria* spp. was greater at site A than at other sites, while the number of individuals of the remaining eight genera was lower at site A than at other sites. Differences between sites B–C and D–F were found for six genera.

The first two axes of the PCA ordination of benthic invertebrate genus composition for 10 sites explained 73.7% of the variation (Fig. 4). Sites B–F were clustered to the right, whereas sites O1, O2 and A were located at the upper left of the ordination diagram. Sites P1 and P2 were located in the lower middle section of the ordination diagram. Cluster analysis of genus composition among the 10 sites divided benthic invertebrates into three groups when the Euclidean distance of 0.5 was selected (Fig. 5). The first group consisted of O1, O2 and A. The second group consisted of three sites (D–F). The third group consisted of four sites (P1, P2, B and C). Axis 1 differed significantly between three groups according to the cluster analysis ( $KW = 7.3$ , d.f. = 2,  $P < 0.05$ , Kruskal–Wallis test) and the difference was detected between the group of O1, O2 and A and

**Table 1.** Riparian (stream width, altitude, forest in buffer zone, residential in buffer zone) and in-stream environmental conditions (EC, pH, DO, water and air temperatures, density of chlorophyll a and c) at six sites and their summary statistics (Friedman test).

Environmental factors	Sampling sites						Friedman test			
							Sites (d.f. = 5)		Months (d.f. = 5)	
	A	B	C	D	E	F	<i>Fr</i>	Pairwise differences	<i>Fr</i>	Pairwise differences
Stream width (m)	5	10	10	11	13	26	—		—	
Altitude (m)	550	330	300	200	130	90	—		—	
Forest in buffer zone (percentage)†	100	100	100	81.3	56.7	58.9	—		—	
Residential in buffer zone (percentage)†	0	0	0	18.7	43.3	41.1	—		—	
Electrical conductivity (EC) (µs/cm)††	55.7 ± 13.7	51.3 ± 7.03	50.7 ± 8.66	42.0 ± 17.40	55.0 ± 7.87	50.0 ± 13.8	11.196*		12.936*	
pH††	7.06 ± 0.290	7.03 ± 0.207	7.17 ± 0.320	7.25 ± 0.451	7.35 ± 0.686	7.68 ± 1.078	2.402		8.735	
Dissolved oxygen (DO) (mg/L)††	10.78 ± 1.790	11.23 ± 2.076	11.04 ± 1.636	11.49 ± 1.757	10.91 ± 1.635	11.25 ± 1.170	8.286		29.600**	Aug. vs. Feb. Aug. vs. Apr. Oct. vs. Feb.
Water temperature (°C)††	11.73 ± 4.775	13.37 ± 5.131	13.88 ± 5.440	14.62 ± 6.071	14.25 ± 5.803	14.25 ± 5.778	17.714**	A vs. E A vs. F	28.095**	Aug. vs. Dec. Aug. vs. Feb.
Air temperature (°C)††	13.55 ± 6.981	17.73 ± 6.737	17.73 ± 6.980	16.62 ± 5.685	16.83 ± 7.442	17.02 ± 8.564	10.584		27.143**	Aug. vs. Dec. Aug. vs. Feb. Oct. vs. Feb.
Density of chlorophyll a (mg/m <sup>2</sup> )††	4.37 ± 3.136	4.40 ± 3.876	4.79 ± 3.281	3.94 ± 2.382	5.84 ± 3.983	5.04 ± 4.700	1.524		13.333*	Oct. vs. Jun.
Density of chlorophyll c (mg/m <sup>2</sup> )††	3.29 ± 2.809	2.66 ± 1.306	1.69 ± 2.519	0.98 ± 1.338	0.55 ± 3.431	0.06 ± 4.079	9.810		13.905*	Dec. vs. Jun.

†The percentage of the area of both buffer zone 250 m in width from each side of the wetted streamline between the sites; ††averaged data per month, Average ± SD; \**P* < 0.05; \*\**P* < 0.01.

**Table 2.** Four indices (number of individuals, number of families, number of genera, diversity index) of the benthic invertebrate assemblages at six sites (A–F) and their summary statistics (Friedman test).

Indices	Sampling sites						Friedman test			
	A	B	C	D	E	F	Sites (d.f. = 5)		Months (d.f. = 5)	
							<i>Fr</i>	Pairwise differences	<i>Fr</i>	Pairwise differences
Number of individuals†	346.8 ± 177.84	566.0 ± 212.33	418.7 ± 169.15	472.3 ± 183.85	411.5 ± 143.08	399.3 ± 188.73	12.762*	A vs. B	16.952**	Oct. vs. Jun. Apr. vs. Jun.
Number of families†	20.1 ± 4.11	23.7 ± 2.94	24.2 ± 5.34	25.2 ± 4.26	20.8 ± 2.04	25.2 ± 4.12	13.538*	—	11.068*	Dec. vs. Jun.
Number of genera†	29.2 ± 7.41	36.3 ± 5.68	35.7 ± 8.21	37.8 ± 7.11	30.0 ± 3.52	36.5 ± 5.61	13.727*	A vs. D	15.814**	Oct. vs. Jun. Dec. vs. Jun.
Diversity index†	2.38 ± 0.280	2.57 ± 0.143	2.40 ± 0.287	2.75 ± 0.302	2.37 ± 0.227	2.59 ± 0.232	15.143**	—	15.238**	Dec. vs. Apr.

†Averaged data per month in each site of five quadrats, Average ± SD; \* $P < 0.05$ ; \*\* $P < 0.01$ .

**Table 3.** Spearman's rank correlations ( $\rho$ ) between four indices (Table 2) and environmental conditions (Table 1).

	Stream width (6)	Altitude (6)	Forest in buffer zone (6)†	Residence in buffer zone (6)†	Electrical conductivity (36)	pH (36)	Dissolved oxygen (36)	Water temperature (36)	Air temperature (36)	Density of Chl. a (36)	Density of Chl. c (36)
Number of individuals (36)	-0.023	0.070	0.106	-0.106	0.120	0.217	0.132	-0.163	-0.130	0.558**	0.508**
Number of families (36)	0.172	-0.166	0.039	-0.039	0.014	0.350*	0.046	-0.061	-0.009	0.232	0.294
Number of genera (36)	0.136	-0.126	0.063	-0.063	-0.063	0.410*	-0.051	0.011	0.084	0.240	0.243
Diversity index (36)	0.176	-0.144	-0.058	-0.058	0.146	0.156	0.078	-0.187	-0.136	0.097	0.239

†The percentage of the area of both buffer zone 250 m in width from each side of the wetted streamline between the sites; (number), sample size; \* $P < 0.05$ ; \*\* $P < 0.01$ .

**Table 4.** Statistical results for two axes of the principal components analysis among six sites and among 6 months, and spearman's rank correlations ( $\rho$ ) between two axes of PCA (Table 4) and environmental conditions (Table 1).

	Friedman test		Stream width (6)	Altitude (6)	Forest in buffer zone (6)†	Residence in buffer zone (6)†	Electrical conductivity (36)	pH (36)	Dissolved oxygen (36)	Water temperature (36)	Air temperature (36)	Density of Chl. a (36)	Density of Chl. c (36)
	Site <i>Fr</i> (d.f. = 5)	Months <i>Fr</i> (d.f. = 5)											
Axis 1	23.238**	4.667	0.640**	-0.661**	0.514**	-0.661**	0.054	0.221	-0.007	0.238	0.152	-0.108	0.330
Axis 2	16.286**	18.952**	-0.551**	0.565**	-0.083	0.565**	0.194	-0.363	0.596**	-0.746**	-0.648**	0.386*	0.165

†The percentage of the area of both buffer zone 250 m in width from each side of the wetted streamline between the sites; (number), sample size; \* $P < 0.05$ ; \*\* $P < 0.01$ .

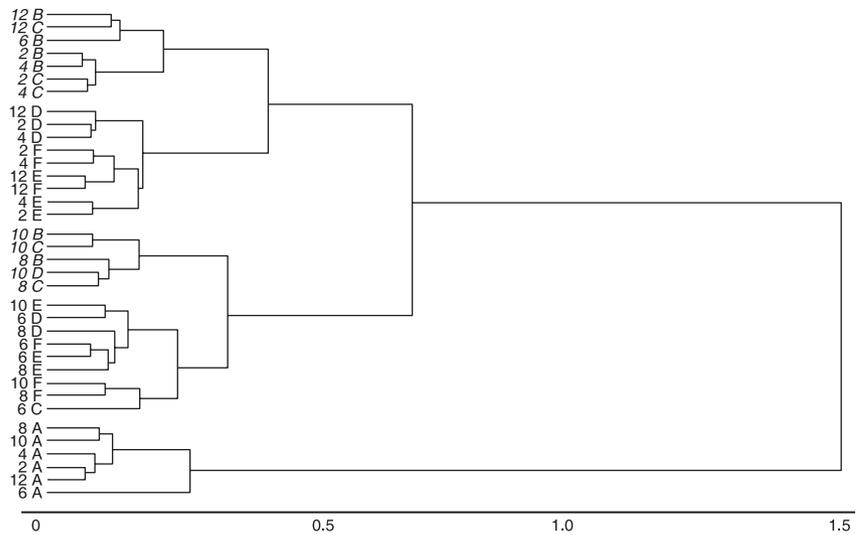
**Table 5.** Abundance of benthic invertebrates at each site and their statistical analysis (Friedman test).

	Sampling sites (n/m <sup>2</sup> )						Friedman test	
	A	B	C	D	E	F	<i>F</i> (d.f. = 5)	Pairwise differences
Arthropoda								
Insecta								
Plecoptera								
<i>Togoperla</i> spp.	46.2	25.6	5.6	1.1	0.4	0.4	24.643***	A vs. D, A vs. E, A vs. F
<i>Paragnetina</i> spp.	0.4	2.9	4.0	0.4	0.4	0.0	12.841*	—
<i>Calineuria</i> spp.	16.2	1.6	1.6	0.0	0.0	0.4	24.130***	A vs. D, A vs. E
<i>Kiotina</i> spp.	2.7	1.1	1.1	0.0	0.0	0.0	12.282*	—
<i>Kamimuria</i> spp.	0.0	35.1	30.0	49.6	20.0	10.4	20.343**	A vs. B, A vs. D
<i>Ostrovus</i> spp.	0.4	5.6	4.9	20.0	5.6	10.7	12.059*	—
<i>Amphinemura</i> spp.	20.4	117.1	15.6	13.3	0.7	0.4	20.120**	B vs. E, B vs. F
<i>Sweltsa</i> spp.	15.6	6.2	11.8	2.7	4.0	4.4	11.571*	—
Ephemeroptera								
<i>Isonychia</i> spp.	0.0	2.7	0.7	36.0	2.9	2.9	20.763**	A vs. D, C vs. D
<i>Baetis</i> spp.	49.6	178.9	87.8	101.1	131.8	63.8	11.555*	—
Trichoptera								
<i>Stenopsyche</i> spp.	0.0	47.8	23.8	70.4	11.8	8.4	22.452***	A vs. B, A vs. D
<i>Uenoi</i> spp.	0.0	20.7	26.0	1.6	0.0	0.0	15.756**	—
<i>Hydropsyche</i> spp.	0.0	34.9	16.2	12.7	2.9	4.0	20.759***	A vs. B, B vs. E
<i>Cheumatopsyche</i> spp.	3.3	41.1	27.3	45.1	6.7	7.8	15.916**	A vs. D
<i>Diplectrona</i> spp.	0.7	27.1	9.3	9.3	6.0	1.1	12.340*	—
<i>Lepidostoma</i> spp.	24.4	1.1	12.2	36.0	23.3	42.9	16.643**	B vs. D
<i>Dolophilodes</i> spp.	0.0	3.3	0.0	1.1	0.0	0.0	11.857*	—
Odonata								
<i>Epiophlebia</i> spp.	10.0	2.2	0.7	0.7	0.4	0.0	15.405**	—
<i>Lanthus</i> spp.	1.8	15.6	10.4	18.2	6.2	6.0	12.601*	A vs. D
<i>Calopteryx</i> spp.	0.7	0.0	0.7	2.9	0.0	0.0	12.042*	—
Megaloptera								
<i>Protohermes</i> spp.	0.0	24.4	4.4	15.1	3.8	3.8	20.508**	A vs. B, A vs. D

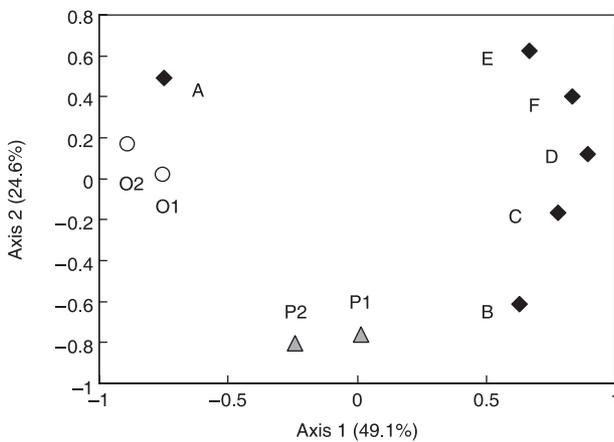
Table 5. Continued

	Sampling sites (n/m <sup>2</sup> )						Friedman test	
	A	B	C	D	E	F	Fr(d.f. = 5)	Pairwise differences
Coleoptera								
<i>Eubrianax</i> spp.	2.6	3.8	4.4	37.3	57.1	44.9	19.059**	A vs. E
<i>Cyphon</i> spp.	0.0	3.8	9.6	5.6	18.2	8.2	11.508*	—
Diptera								
<i>Asuragina</i> spp.	1.1	1.6	4.9	15.6	3.3	2.9	13.636*	B vs. D
<i>Atherix</i> spp.	26.0	1.1	0.0	0.0	0.0	0.0	19.700**	—
<i>Hexatomini</i> Gen. spp.	7.3	3.3	1.1	2.2	4.9	12.2	14.22*	C vs. F
<i>Antocha</i> spp.	1.8	2.9	4.0	21.1	15.1	11.1	14.923*	—
<i>Nagatomyia</i> spp.	2.7	0.0	0.0	0.0	0.0	0.0	15.000*	—
Crustacea								
Amphipoda								
<i>Rivulogammarus</i> spp.	0.0	1.8	0.4	0.0	0.0	0.4	12.857*	—
Decapoda								
<i>Geothelphusa</i> spp.	4.4	8.2	4.9	0.0	0.7	0.0	17.488**	—
Annelida								
Hirudinoidea								
Pharyngobdellida								
<i>Erpobdella</i> spp.	0.0	0.0	0.0	1.6	2.7	2.7	12.569*	—
Plathelminthes								
Turbellaria								
Tricladida								
<i>Phagocata</i> spp.	4.4	4.0	0.7	1.6	0.0	2.2	12.189*	—

Density in each site per a month are indicated; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Fig. 3.** Cluster analysis of similarities (Spearman's correlation coefficients) in benthic invertebrate genera among 36 samples (six sites and six sampling months) using the Ward method and Euclidean distances.



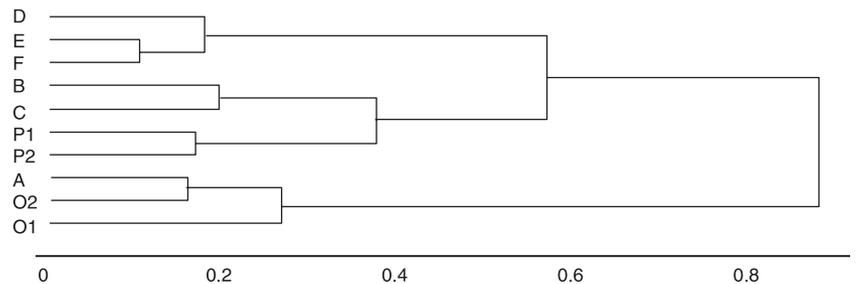
**Fig. 4.** Principal components ordination diagram of benthic invertebrate genus composition. Letter, sampling site; ◆, A–F; ○, O1, O2; ▲, P1, P2.

the other two groups. Axis 2 also differed significantly between three groups according to the cluster analysis ( $KW = 6.7$ , d.f. = 2,  $P < 0.05$ , Kruskal–Wallis test) and the difference was detected between the group of P1, P2, B and C and the other two groups.

Abundance in three groups was significantly different in 15 genera and pairwise group differences were detected for six genera (Table 6). The numbers of individuals of *Ostrovus*, *Ephemera*, *Eubrianax*, and *Cyphon* spp. were highest in the group comprising D, E and F. The numbers of individuals of *Baetis* and *Hydropsyche* spp. were highest in the group comprising P1, P2, B and C.

**Discussion**

Obvious differences between benthic invertebrate assemblages of site A and the other sites (B–F) were shown by PCA. Stream width, altitude and surrounding land use were mutually highly correlated, and were considered as factors defining the character of riparian condition at each site. Axis 1 of the PCA was correlated with these factors, but not with in-stream environmental factors (water temperature, conductivity, pH and DO) and air temperature. Hence, axis 1 may be explained by differences in riparian condition. The preference of environmental conditions among benthic invertebrates differed depending on the genus (Merritt & Cummins, 1996). In-stream environmental differences were considered as likely causes of site differences in the benthic invertebrate assemblage. However, site difference in the benthic invertebrate assemblage was not correlated with in-stream environmental factors. The numbers of individuals, families, and



**Fig. 5.** Cluster analysis of similarities (Spearman's correlation coefficients) in benthic invertebrate genera among 10 sites using the Ward method and Euclidean distances.

**Table 6.** Abundance of benthic invertebrates at each group of 10 sites and their statistical analysis (Kruskal–Wallis test).

	Sites D, E, F (n = 3)	Sites B, C, P1, P2 (n = 4)	Sites O1, O2, A (n = 3)	KW test			
				(d.f. = 2) H	Pairwise differences		
				(D, E, F) vs. (B, C, P1, P2)	(D, E, F) vs. (O1, O2, A)	(B, C, P1, P2) vs. (O1, O2, A)	
Arthropoda							
Insecta							
Plecoptera							
<i>Ostrovus</i> spp.	46.7	6.7	0.0	6.636*	—	*	—
<i>Nemoura</i> spp.	0.0	0.6	8.9	6.884*	—	—	—
Ephemeroptera							
<i>Ephemera</i> spp.	25.2	3.3	16.3	6.192*	*	—	—
<i>Baetis</i> spp.	98.5	159.4	39.3	6.787*	—	—	*
<i>Paraleptophlebia</i> spp.	59.3	4.4	57.8	6.644*	—	—	—
Trichoptera							
<i>Stenopsyche</i> spp.	75.6	70.0	0.0	6.130*	—	—	—
<i>Rhyacophila</i> spp.	5.2	1.1	0.0	6.299*	—	—	—
<i>Goera</i>	6.7	0.0	0.0	8.670*	—	—	—
<i>Ueno</i>	2.2	107.8	3.7	6.987*	—	—	—
<i>Hydropsyche</i> spp.	21.5	49.4	0.0	6.457*	*	—	—
Odonata							
<i>Davidius</i> spp.	3.0	0.6	0.0	6.449*	—	—	—
Coleoptera							
<i>Eubrianax</i> spp.	77.0	7.2	1.5	6.587*	—	*	—
<i>Cyphon</i> spp.	21.5	3.9	0.0	7.517*	—	*	—
Diptera							
<i>Atherix</i> spp.	0.0	0.6	20.7	7.509*	—	—	—
<i>Antocha</i> spp.	37.0	2.8	0.7	6.368*	—	—	—

Density on December in each group is indicated; \* $P < 0.05$ .

genera differed significantly among sites along Kuroson Stream. EC and water temperature also differed significantly among sites along the stream. In spite of this, the number of individuals, family and genera were not highly correlated with these in-stream environmental factors.

Cluster analyses also showed that the benthic invertebrate assemblage at site A differed from all other sites. Differences between site A and other sites were detected in other indices such as abundance and the number of genera. Although site differences were observed along Kuroson Stream, no measured environmental factors explained the large difference between site A and the other sites. Site A was in old growth natural forest and other sites were in a mixture of broadleaved and coniferous forest. Benthic invertebrate assemblages vary depending on the watershed forest types, and species specific to each of forest types inhabit (Yoshimura & Maeto, 2006). The difference between site A and other sites may have been caused in part by the differences in riparian and watershed vegetation type. Various types of disturbance and physical conditions create heterogeneous substrates that act as patches for benthic invertebrate populations in the stream (Reice, 1994). Miserendino (2001) reported that habitat heterogeneity was the strongest predictor of macroinvertebrate assemblages and could be used to predict species richness at the landscape scale. Substrate conditions of these sites in this study might be different depending on the watershed forest type. Recognition of the variability of riparian, watershed

and in-stream landscapes is necessary to understand difference in benthic invertebrate assemblages. Abundances for 10 of 14 genera at site A were different from those of other sites. At site A, there were more individuals of *Togoperla* spp. and *Calineuria* spp. Abundances of the other eight genera were lower at site A. *Togoperla* spp. and *Calineuria* spp. are considered as inhabitants of upper reaches, whereas the other eight genera are considered as inhabitants of upper to lower reaches (Kawai & Tanida, 2005). These obvious differences may have determined in part the classification of benthic invertebrate assemblages into two groups (site A and the other sites).

Axis 2 of the PCA was explained by site and seasonal differences, and was correlated with almost all the environmental factors measured. Cluster analysis of sample similarities also revealed differences between seasons (December–April and June–October). The number of individuals, families, and genera also differed significantly among six sampling months. In-stream environmental factors varied seasonally, and they clustered into two groups of sampling months. Univoltinism, seasonal drift, and habitat selection may account for seasonal differences in the benthic invertebrate assemblage (Merritt & Cummins, 1996). The difference of emergence season of each species may have resulted in the absence of larvae in the water during some sampling months. Seasonal differences in benthic invertebrate assemblages may be in part a consequence of the seasonal change of in-stream environmental factors, but the most obvious

thing would be a consequence of different activity patterns of the species over the year.

Cluster analysis of sample similarities showed that the benthic invertebrate assemblages along Kuroson Stream were also different between sites B–C and sites D–F. Pairwise abundance differences among five sites of B–F were detected only between the sites B–C and D–F. At site D, abundances were often higher or lower than those of other sites. The difference of riparian vegetation would lead to higher abundances in *Togoperla* spp., *Calineuria* spp. and *Sweltsa* spp. and lower abundances in *Paragnetina* spp., *Ostrovus* spp. and *Uenoi* spp. at site A than site B and C. The abundance of these genera was also different between site C and site D. Besides, in *Eubrianax* spp., *Antocha* spp., *Geothelphusa* spp. and *Erpobdella* spp. although there was no abundant difference between site A and site B–C, difference of abundance between site C and site D was apparent. Since tolerance or preference of water pollution of each genus is different, abundance difference would be revealed. Riparian condition were clustered into two groups (A–C and D–F), and there were no residential areas at sites A through site C. Increase in occurrence of residential areas may have an effect on the in-stream condition causing the differences in invertebrate benthos between sites B–C and sites D–F.

When the data for sites O1, O2 and P1, P2 were included in PCA and cluster analysis, the benthic invertebrate assemblage of site A was similar to those of sites O1–O2. Sites D–F along Kuroson Stream comprised another cluster that differed from the cluster of B–C and P1–P2. Benthic invertebrates are thought to be carried by flowing water from tributaries into the main stream habitat (O'Hop & Wallace, 1983). Individuals of *Gammarus* and *Baetis* can drift >30 m/day (Waters, 1965). Most benthic invertebrates can move upstream, but at < 8 m/day (Elliott, 1971). When gaps in the benthos are created by disturbance, benthic invertebrates that inhabit the surrounding substrate colonise the available space; taxon richness recovers in 3 days, and density recovers in 1 month (Hayashi, 1991; Matthaei *et al.*, 1996). This means that benthic invertebrates are able to settle after moving to new suitable substrate. Thus, benthic invertebrates carried from tributary streams appear to affect the assemblage in the main stream. Water from P1, P2, and O2 flowed into the upper reaches of sites B, D, and E, respectively. Accordingly, the benthic invertebrate assemblages of the 10 sites were expected to be mingled. However, this was not observed in the PCA or the cluster analysis. Sites P1, P2, B, and C formed one cluster. It seems to show these sites are nested. However, site D, which is considered to be affected by the assemblage from P2, was excluded from this cluster. The benthic invertebrate assemblage in site O2 was also different from that in site E. Although benthic invertebrates move upstream and drift downstream, the range of the movement appears to have been insufficient to homogenise assemblages among sites.

Riparian vegetation of sites B–F comprised a mixture of broadleaved and planted coniferous trees, that of sites O1, O2 and A was natural old-growth forest, and that of sites P1 and P2 was planted coniferous forest. Water quality and benthic invertebrates differ among native forest, pine forest and pasture land (Quinn *et al.*, 1997). Stream water quality in the forest also varies with forest type (Friebert, 1997; Friberg *et al.*, 1997). Hemlock

forests support more benthic invertebrate taxa than mixed hardwood forests (Snyder, 2002). Benthic invertebrates differ between natural broad-leaved and the planted coniferous forests (Yoshimura & Maeto, 2006). Although the type of riparian vegetation was different between B–C and P1–P2, samples fell into a single cluster in our multivariate analysis. Riparian vegetation at sites B–C and P1–P2 consisted of Japanese cedar plantations. In addition, human settlements begin at the lower reaches of site C. Stream water quality varies according to surrounding land use (*viz.*, forest, urban and residential areas; Sweeney, 1992; Hall *et al.*, 2001). The variation in riparian vegetation type and the occurrence of human settlement likely caused these differences in benthic invertebrate assemblages.

In New Zealand, streams flowing through pastures are dominated by chironomids, snails and worms. Several mayfly and stonefly taxa are common in pine and natural forest streams, but absent in pasture streams (Quinn *et al.*, 1997). They explain this pattern by differences among invertebrates in the tolerance of organic and enrichment pollution. Athericidae and Taeniopterygidae are dominant in natural broad-leaved forest, and Stenopsychidae are dominant in planted coniferous forests, although EC and pH differ little between these forest types in Japan (Yoshimura & Maeto, 2006). In this study, individuals of *Ostrovus*, *Eubrianax* and *Cyphon* spp. were more numerous in the group comprising D, E, and F. Habitats that have moderate stream velocity occur more frequently in the lower reaches than in the upper reaches. Consequently, preference for moderate velocity in these genera may lead to increased abundance in the group comprising D, E, and F. *Baetis* and *Hydropsyche* spp. tend to inhabit coniferous forest waters (Yoshimura & Maeto, 2006). Japanese cedar is also planted at sites B–F. This might lead to increased abundances of *Baetis* and *Hydropsyche* spp. in the group comprising P1, P2, B and C and lower abundance in the group comprising O1, O2 and A. Riparian and in-stream conditions are intricately intertwined and they form the character of each site. Habitat preference also varies among genera. Consequently, even if these benthic organisms drift from tributaries to the main stream, they may crawl back upstream to the tributary if the new habitat conditions are not satisfactory.

Coniferous forest in the tributaries had been cut and afforested. We do not know the vegetation of these tributaries before the cutting and afforestation. Site A and site O2 had the similar assemblages of benthic invertebrate. It might be proper to consider that coniferous tributaries were covered with old-growth natural forest before cutting and afforestation by humans, and the benthic invertebrate assemblage in the coniferous tributaries would be similar with that in old-growth natural forest. In the course of vegetation shift from old-growth natural forest to coniferous forest, some species may move to other suitable habitats such as tributaries of A and O2, some species may remain, some species may extinct, and other species may new enter this habitat.

Slight impact of human residence affects on the benthic invertebrate assemblage, perhaps accounting for the benthos differences between the forests that have been used by humans (*e.g.*, forestry, camping and mountaineering) and those that never has. Human activity in the forested area surely affects the benthic invertebrates. However, since humans are also a part of

nature, they have a right to be active in the forest area. For the sake of reducing the unnecessary disturbance and keeping the human impact to a minimum on benthic invertebrate, we may need some kind of guideline that shows the limit of human impact on benthic invertebrate assemblage, which might lead the sustainable coexistence with nature.

Riparian forest type may be one of the most important variables affecting benthic invertebrate assemblages. The large difference in the benthic invertebrate assemblage between site A and the other sites along a stream is likely due to differences in the composition of the surrounding vegetation. Taking into account larval drift, the differences in the environmental conditions among sites, and the early seasonal progress of larvae in the lower reaches, the similarity of benthic invertebrate assemblages among adjacent sites and seasons is expected. However, there was a clear difference between sites B–C and D–F in the benthic invertebrate assemblage, which was very likely caused by the existence of a residential area. This study indicates that human settlement, even at a small scale, does impact the benthic invertebrate assemblage. Benthic invertebrate assemblages in tributaries were different from those in the main stream and the difference was related to surrounding vegetation. The drift of benthic invertebrate from tributaries does not seem to counterbalance the differentiation of benthic invertebrate assemblages by human activity. Of course, some species and some individual may drift and settle in other sites, but considering as assemblages, the appearance of benthic invertebrate assemblage might be stable as far as the surrounding environment is similar. Seasonal changes in benthic invertebrate assemblages occurred independently at each site. So, each assemblage of benthic invertebrates that inhabit a particular site may not change and are considered to be active and vary seasonally across a certain range where the impact from surrounding environment is at a similar level.

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