

**Effect of environmental factors on the abundance of riffle beetles (Coleoptera: Elmidae) and co-inhabit aquatic insects within a reach scale, in Japan**

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Running Head: Benthic structure and riffle beetle

## Abstract

Riffle beetles (Coleoptera: Elmidae) are freshwater insects, and both adult and larval stages primarily inhabit running water. Therefore, environmental factors such as current velocity and substrate characteristics are crucial for the habitat of elmid beetles. The effects of substrate size structure, water depth, current velocity, and distance from terrestrial areas were evaluated for the adult and larval abundance of three common riffle beetles, *Stenelmis nipponica* Nomura, *Zaitzevia awana* (Kono), and *Zaitzeviaria brevis* (Nomura), and other cohabiting aquatic insects in a middle reach of the Mukogawa River, western Japan. The substrate size structure was summarized using principal component analysis, and the effects of environmental factors were analyzed using best subsets multiple regression. In summer (August), the three elmid species tended to be more abundant in the shallow quadrats of coarse-grained substrates, and substrate preference differed among species and stages. Current velocity and proximity to the terrestrial area were not significant for *S. nipponica* and *Z. brevis*, whereas adult *Z. awana* preferred faster-flowing currents and needed to the distance from the terrestrial areas. In winter (December), only the substrate size structure affected the abundance of adult elmid beetles. Although elmid beetles require highly sensitive habitats, they are easily surveyed. Therefore, they are convenient indicators of riverbed environments.

## Implications for insect conservation

Preference of substrate sand size as their habitat differ among stages and seasons. Diverse substrate condition would be needed to conserve elmid beetles.

**Keywords** Current velocity · Mukogawa River · Elmidae · Riverbed sediment · Substrate size structure · Water depth

## Statements and Declarations

## Conflict of interest

The authors declare that they have no conflict of interest.

43     **Informed consent**

44     Informed consent was obtained from all individual participants included in the study.

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51     **Author Contribution**

52     Dr. J. Fujiwara did the study of conceptualization, investigation, visualization, writing original draft, review  
53     & editing. Dr. K. Maeto did the study of analysis, writing original draft, review & editing. Dr. M. Yoshimura  
54     did the study of conceptualization, analysis, writing original draft, review & editing.

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## Introduction

The structure of aquatic insect assemblages in rivers differs depending on physicochemical conditions, such as temperature, channel stability, current velocity, and water pathways (Burgherr and Ward 2001; Collier 2004). The density of riparian vegetation, woody debris in the river, the dissolved oxygen concentration of the river, and other water qualities also affect the abundance and species richness of aquatic insects (Quinn and Hickey 1990a, b; Olsen and Townsend 2003). The benthic substrate is a major environmental factor that affects inhabitation by aquatic insects (Quinn and Hickey 1990a, b; Burgherr and Ward 2001; Olsen and Townsend 2003; Collier 2004). Changeable heterogeneous substrates are created by various types of disturbance that occur in rivers, and they can act as habitat patches for various kinds of aquatic insects (Reice 1994). Therefore, a high diversity of aquatic insects can exist on heterogeneous substrates, which provide ample food availability (Godbout and Hynes 1982). Habitat heterogeneity is a good indicator of the species richness of aquatic insects (Yoshimura 2012).

Most Elmidae (Coleoptera) species are small freshwater insects that inhabit running water in both the adult and larval stages (Brown 1987; Ogata and Nakajima 2006; Elliot 2008; Jäch and Balke 2008; Hayashi and Sota 2019; Nair et al. 2019). In Japan, they are abundant in the running streams of mountainous areas (Yoshitomi et al. 1999; Shiyake and Beetle Team of Yamatogawa River Research Group 2008; Tominaga et al. 2012). They cling to plant bodies, stones, and woody debris on benthic substrates with their tarsal claw (Tominaga et al. 2012). Larval and adult respiration are performed through tracheal gills and a plastron system of hair-like setae (Thorpe 1950; Hayashi and Sota 2019). Therefore, they require well-oxygenated fast-running water containing fewer surfactants (Ogata 2000; Elliott 2008).

Various environmental factors, such as substrate, vegetation, current velocity, and detritus, also affect the existence of elmidae beetles, and their effects differ depending on the species (Malmqvist and Sjöström 1983; Dietrich and Waringer 1999; Lloyd and Sites 2000). Elmidae beetles seem to have higher sensitivity to the changes in environmental conditions (Aguilera-Giraldo and Vásquez-Ramos 2019; González-Córdoba et al. 2020). Current velocity is an important factor for some elmidae species, whereas substrate characteristics

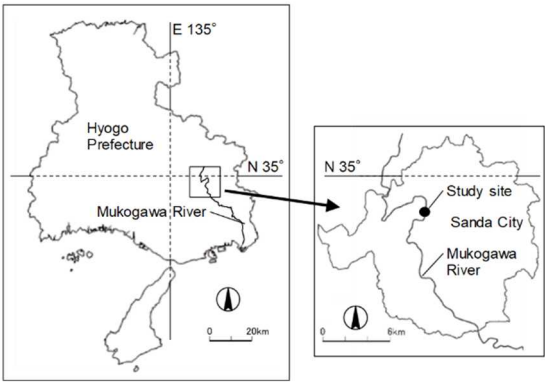
are the major determinants for other species (Lloyd and Sites 2000). Previous studies have identified the environmental factors necessary for suitable elmid beetle habitat based on the basin scale relationship between the number of beetles and their environmental factors. However, each environmental condition in the river is spatiotemporally mosaic in nature. Environmental factors such as current velocity, substrate sand, and water depth are not stable, but change from moment to moment at the reach scale. Even a distance of a few tens of centimeters can create a very different environment.

It can be assumed that the effects of benthic structures vary from species to species, but that the differences in developmental stages are greater than the differences between species. Therefore, clarification of the relationship between the number of individuals and environmental factors within a reach scale is necessary to understand the spatiotemporal movement of elmid beetles and aquatic insects. However, the difference in the effects of environmental factors on adults and larvae of elmids has not also been clarified. In this study, the within-reach-scale effects of the substrate size structure, water depth, current velocity, and distance from terrestrial areas on the abundance of aquatic insects were evaluated by sampling quadrats in a mesh grid in a lowland river of Japan. The distribution pattern of three common riffle elmid beetles, *Stenelmis nipponica* Nomura, 1958, *Zaitzevia awana* (Kono, 1934), and *Zaitzeviaria brevis* (Nomura, 1958) throughout their life cycle was investigated. The habitat preferences of other aquatic insects were also assessed to compare them with elmids.

# Materials and methods

## Study site

The study was conducted in a middle reach of the Mukogawa River, Sanda Basin, Hyogo Prefecture, western Japan (Fig. 1). The Mukogawa River flows from the eastern part of Hyogo Prefecture,



Sasayama Basin (altitude of approximately 200 m), into Osaka Bay. The total length of the river is 69.3 km. This study was conducted approximately 50 km from the river mouth (34.970556N, 135.196111E, altitude 160 m; Kobayashi 2002). There is no water storage in the upper reaches of the river. The river slope at the study site was approximately 1/200.

The study site was surrounded by paddy fields approximately 100 m in width, behind which were second-growth broadleaved forests (Fig. 2). The river width at the study site was approximately 22 m and the riverbanks were



constructed with a concrete wall. There were several terrestrial areas within the river formed by the deposition of gravel where *Salix* spp. or *Phragmites* spp. grew thickly. There was no flooding of the Mukogawa River in 2009.

#### Measurement of environmental variables

Field surveys were conducted between early morning (5 am) and evening (6 pm) in summer (August 16–21) and winter (December 1–3) 2009. It was fine or cloudy, except for a rainy day on December 3. Quadrats (25 cm × 25 cm) for sampling were set at 2 m intervals from the right to left banks and from the upper to lower reaches of the river.

The environmental variables were measured in the middle of each quadrat when aquatic insect sampling was conducted. Both the temperature and dissolved oxygen were measured using a dissolved oxygen meter (OM-51-2, HORIBA, Kyoto, Japan). Current velocity was measured just above the riverbed using an anemometer (FP101, Global Water, CA, USA). The water depth and distance from the center of each quadrat to its closest terrestrial area were measured using a scale and tape measure.

From each quadrat, 1,000 ml of riverbed substrate was collected using a plastic cup. The substrate was divided into eight size classes (>31.5 mm, 16.0–31.5 mm, 8–16 mm, 4–8 mm, 2–4 mm, 1–2 mm, 0.5–1.0 mm, 0.25–0.50 mm) using testing sieves (Tokyo Screen, Tokyo, Japan). The weight of the substrate was

measured using an electronic balance (GF-6100, A&D, Tokyo, Japan) and the proportion of the weight of each size class to the total substrate was calculated.

#### Sampling of benthic invertebrates

A total of 64 quadrats were used for sampling in August. Because the water level was lower in winter, 51 quadrats were used for sampling in December. The sampling of aquatic insects was conducted using a D-frame net (IS40-1W, HOGA, Kyoto, Japan) with a fine mesh net (0.3 mm, HOGA, Kyoto, Japan). The river sediment inside the quadrat was disturbed after setting the net downstream of the quadrat, and the material flowing into the D-frame net was collected. The material was transferred to a white plastic tray, and invertebrates were picked up. They were stored in screw tubes containing 75 % ethanol, transported to the laboratory, identified using a binocular stereo microscope (SMZ 645, Nikon, Tokyo, Japan), and counted. Adults and larvae of the three elmids species, *S. nipponica*, *Z. awana*, and *Z. brevis*, and larvae of other aquatic insects whose total sample number of individuals was more than ten individuals, were used for statistical analysis in August. In December, only adult samples of the three elmids beetles were used for analysis. Voucher specimens were deposited at the Museum of Nature and Human Activities, Sanda, Hyogo Prefecture, Japan.

#### Statistical analyses

Statistical analyses were performed for the summer (August) and winter (December) datasets separately using IBM SPSS Statistics version 25. Correlation-based principal components analysis (PCA) was conducted to reduce the dimensionality of the eight substrate size classes. Principal components with eigenvalues greater than 1.0 and three other environmental variables were used for the correlation analyses.

The square root of the transformed number of insects ( $\sqrt{n + 0.5}$ ) in each quadrat was calculated. Pearson's correlation coefficient was calculated for the number of aquatic insects, PCA axes, and environmental variables. Principal components and other environmental variables were used for the

regression analyses. Best subsets multiple regression analyses were conducted to demonstrate the effects of environmental variables on the transformed value of insects. The best subsets of environmental variables were then selected based on the Akaike information criterion corrected for small sample sizes.

To determine the structure of the species assemblages collected in August, a principal component analysis (PCA) was conducted on the square root transformed number of insects in each quadrat. Pearson's correlation coefficient was calculated between the main PCA axes and the environmental variables. *Zaitzevia awana* was not included in this study because of its scarcity.

## Results

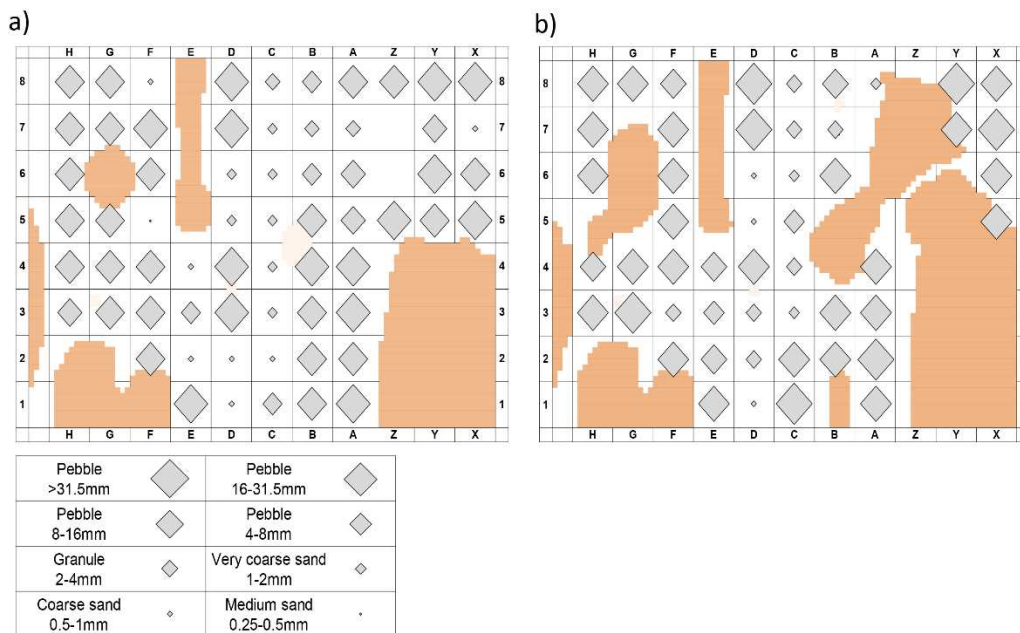
### Environmental variables

The water temperature in each sampling quadrat was  $25.43 \pm 2.33$  °C (mean  $\pm$  SD, n = 64) in August and  $10.33 \pm 1.96$  °C (n = 51) in December. The dissolved oxygen in each sampling quadrat was  $8.99 \pm 1.00$  mg/L (n = 64) in August and  $11.25 \pm 0.94$  mg/L (n = 51) in December. Because sampling was conducted between early morning (5 am) and evening (6 pm), diurnal variations in water temperature and dissolved oxygen were large. Therefore, these factors were excluded from the analysis. Other environmental variables in the sampling quadrats in August and December are listed in Table 1. Substrate size class account for the greatest proportion of riverbed substrate was also indicated in Figure 3. The coefficients of variation for each river substrate size class were larger in August than in December. The average values of water depth and current velocity were larger, but less variable, in August than in December.

Table 1 Summary of the environmental variables.

	August (N = 64)					December (N = 51)				
	Mean	SD	CV	Min.	Max.	Mean	SD	CV	Min.	Max.
Weight proportion to total substrate										
Pebble >31.5mm	0.163	0.199	1.220	0.00	0.83	0.103	0.090	0.867	0.00	0.35
Pebble 16-31.5mm	0.174	0.112	0.642	0.00	0.38	0.150	0.068	0.455	0.00	0.29
Pebble 8-16mm	0.136	0.059	0.435	0.01	0.31	0.143	0.025	0.177	0.08	0.20
Pebble 4-8mm	0.115	0.065	0.567	0.01	0.46	0.133	0.028	0.209	0.06	0.21
Granule 2-4mm	0.112	0.061	0.542	0.00	0.31	0.140	0.072	0.512	0.09	0.59
Very coarse sand 1-2mm	0.124	0.063	0.511	0.01	0.32	0.124	0.032	0.259	0.06	0.20
Coarse sand 0.5-1mm	0.118	0.062	0.528	0.02	0.34	0.117	0.031	0.263	0.05	0.19
Medium sand 0.25-0.5mm	0.059	0.039	0.672	0.01	0.22	0.089	0.021	0.234	0.04	0.16
Water depth (cm)	20.7	12	0.558	2	50	12.5	9	0.718	1	32
Current velocity (m/s)	0.365	0.25	0.689	0.00	1.21	0.259	0.29	1.119	0.00	1.04
Distance from terrestrial area (cm)	198.2	125.6	0.634	25.0	500.0	n/a	n/a	n/a	n/a	n/a





#### Substrate size structure

Three principal components with eigenvalues greater than 1.0 were extracted from eight substrate size variables in both August and December (Table 2). The first three axes of the PCA ordination of substrate sand size explained 81 % and 86 % of the total variation in August and December, respectively. The first principal component (PC1) showed a strong contrast between large pebbles over 31.5 mm and granules and sand under 4 mm (Table 2). PC2 in August and PC3 in December showed a similar contrast, with fewer large pebbles over 31.5 mm and more small pebbles (8–16 mm). PC3 in August and PC2 in December tended to show similar weak contrasts, with fewer small pebbles (4–8 mm) and more large pebbles (16.0–

31.5 mm) in August,

and fewer granules

(2–4 mm) and more

pebbles (8–16 mm)

in December. More

sand (0.25–0.50 mm) was observed in August.

PC1 showed a negative correlation with current velocity in both August and December but was not

Table 2 Eigenvalues (> 1.0) from principal component analyses of the weight proportions of substrates.

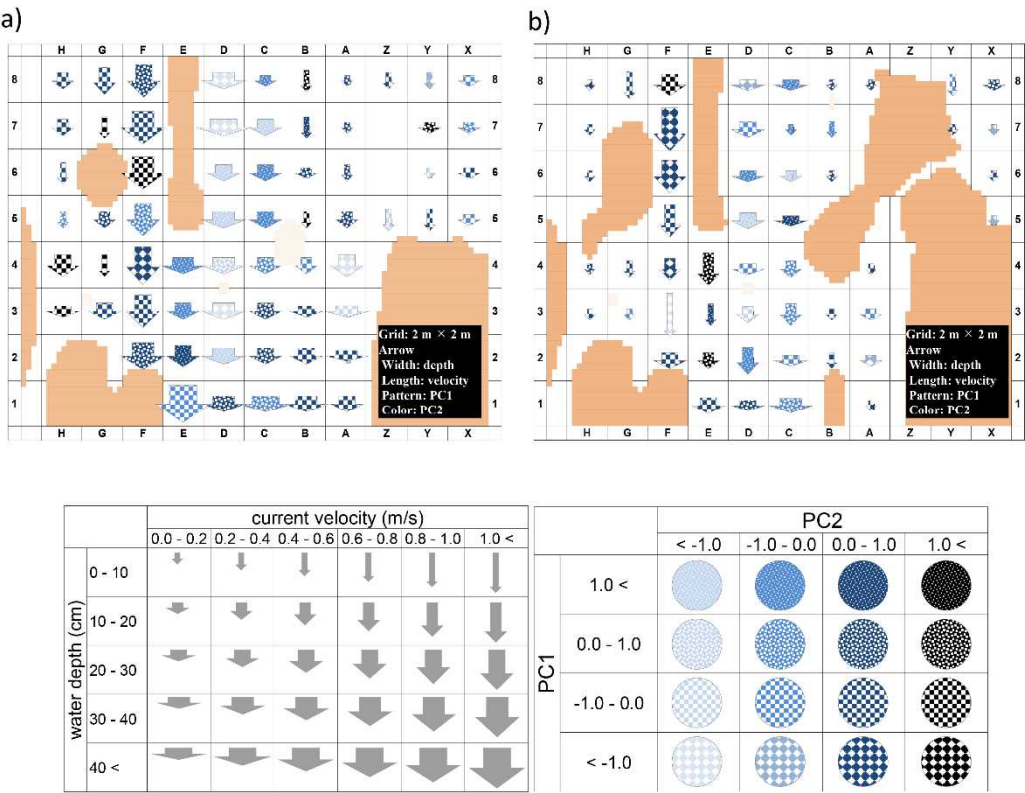
	August			December		
	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalue	3.521	1.857	1.080	4.297	1.476	1.076
Proportion of variance explained	0.440	0.232	0.135	0.537	0.185	0.135
Cumulative proportion of variance explained	0.440	0.672	0.807	0.537	0.722	0.856
Pebble >31.5mm	-0.71	-0.68	-0.16	-0.76	0.06	-0.53
Pebble 16-31.5mm	-0.57	0.63	0.38	-0.73	0.37	0.15
Pebble 8-16mm	-0.07	0.90	0.06	0.28	0.76	0.49
Pebble 4-8mm	0.48	0.33	-0.73	0.80	0.32	0.15
Granule 2-4mm	0.83	0.09	-0.25	0.16	-0.81	0.55
Very coarse sand 1-2mm	0.88	-0.05	0.17	0.92	-0.02	-0.11
Coarse sand 0.5-1mm	0.80	-0.11	0.33	0.92	-0.01	-0.25
Medium sand 0.25-0.5mm	0.60	-0.24	0.42	0.87	0.01	-0.37

significant. PC2 in August showed a significant negative correlation with water depth. PC3 in August showed a significant negative correlation with the distance from the terrestrial area. PC3 in December showed a significant positive relationship with current velocity (Table 3). In August, the water depth and current velocity were positively correlated and seemed to decrease with distance from the closest terrestrial area. The type of substrate (PC1, PC2), current velocity, and water depth in each quadrat in August and December are shown schematically in Fig. 4.

Table 3 Pearson's correlation coefficients between environmental variables in August (n = 64) and December (n = 51).

			Water depth	Current velocity	Distance from terrestrial area
August	Principal components of substrate	PC1	0.018	-0.168	0.205
		PC2	-0.248*	0.198	0.054
		PC3	-0.025	0.104	-0.286*
	Water depth		-	0.261*	-0.215ms
	Current velocity		0.181	-	-0.303*
December	Principal components of substrate	PC1	0.162	-0.203	-
		PC2	-0.047	-0.069	-
		PC3	-0.244	0.457**	-
	Water depth		-	0.181	-

ms P<0.1, \* P<0.05, \*\* P<0.01



Relationship between collected insects and environmental factors

Pearson's correlation coefficients between insect number and environmental variables (Table 4) showed that all correlations with PC1 were negative and significant in some species. Correlation with PC2 was significant in *Z. brevis* and the Sericostomatid *Gumaga* sp. larvae in August. The correlation with PC3 was

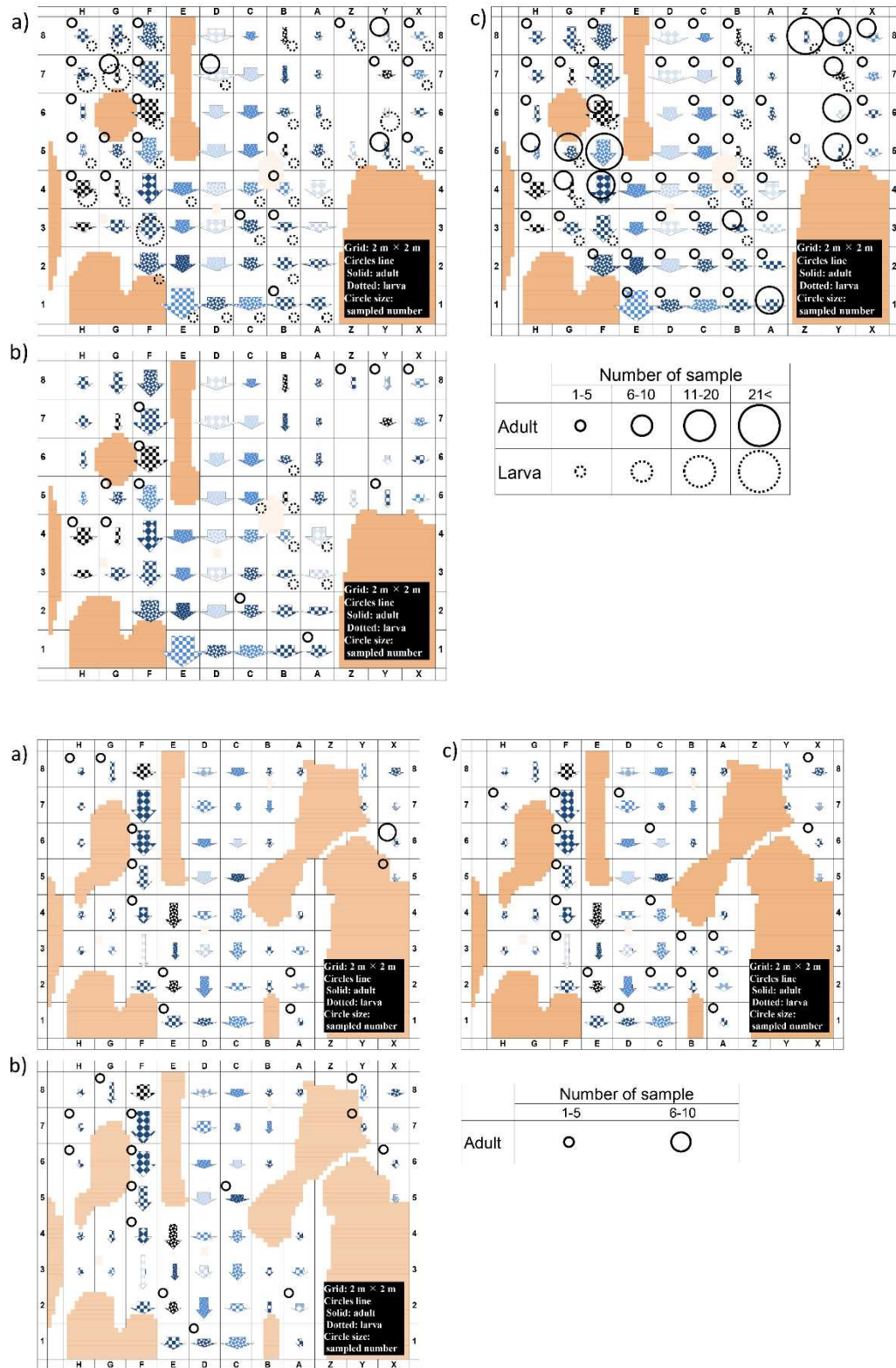
Table 4 Number of insects collected in 25 cm x 25 cm quadrats and associated Pearson's correlation coefficients with environmental variables.

Month / Stage / Taxon	Mean	SD	Med.	Min.	Max.	Total indivs.	Substrate			Water depth	Current velocity	Distance from terrestrial area
							PC1	PC2	PC3			
In August (N = 64)												
Adults												
Coleoptera												
Elmidae												
<i>Stenelmis nipponica</i>	1.1	2.1	0	0	9	73	-0.344**	0.077	0.161	-0.231ms	0.166	-0.045
<i>Zaitavia awana</i>	0.3	0.7	0	0	3	19	-0.185	0.159	0.182	-0.064	0.290*	0.071
<i>Zaitaviaria brevis</i>	4.5	5.2	3	0	24	285	-0.302*	0.176	0.144	-0.276*	0.113	0.050
Larvae												
Coleoptera												
Elmidae												
<i>Stenelmis nipponica</i>	2.1	2.9	1	0	14	135	-0.376**	0.231ms	-0.052	-0.295*	0.185	-0.135
<i>Zaitavia awana</i>	0.1	0.4	0	0	2	9	-0.060	-0.070	-0.154	-0.073	-0.165	-0.063
<i>Zaitaviaria brevis</i>	0.5	1.1	0	0	5	35	-0.123	0.376**	-0.012	-0.308*	0.093	0.078
Psephenidae												
<i>Eubrianax granicollis</i>	0.9	1.4	0	0	6	57	-0.292*	0.099	0.020	-0.359**	-0.017	0.169
<i>Eubrianax ramicornis</i>	9.3	10.9	5.5	0	47	596	-0.465***	0.234ms	0.182	-0.312*	0.158	-0.146
<i>Mataeopsephus japonicus</i>	0.8	1.1	0	0	5	49	-0.266*	-0.140	0.278*	-0.060	0.180	0.091
Ephemeroptera												
Baetidae												
<i>Baetis</i> spp.	0.7	1.3	0	0	6	44	-0.081	-0.036	0.152	-0.260*	0.293*	0.000
Heptageniidae												
<i>Ecdyonurus yoshidae</i>	1.1	1.7	0	0	7	72	-0.344**	-0.142	-0.030	-0.254*	-0.246*	-0.058
Leptophlebiidae												
<i>Choroterpes altioculus</i>	2.2	2.6	1	0	11	139	-0.274*	0.182	-0.045	-0.487***	-0.249*	-0.067
Polymitarcyidae												
<i>Ephoron shigae</i>	0.6	1.3	0	0	5	40	-0.363**	-0.147	-0.135	0.069	-0.195	0.093
Plecoptera												
Perlidae												
<i>Neoperla</i> sp.	0.9	1.3	0	0	5	56	-0.157	0.152	0.064	0.252*	0.546***	-0.096
Trichoptera												
Hydropsychidae												
<i>Hydropsyche orientalis</i>	1.1	2.2	0	0	11	70	-0.282*	0.229ms	0.184	-0.140	0.530***	-0.158
<i>Macrostemum radiatum</i>	0.7	1.9	0	0	10	45	-0.193	0.131	0.068	-0.032	0.503***	0.034
Sericostomatidae												
<i>Gumaga</i> spp.	8.4	9.6	6	0	57	537	-0.554***	0.271*	0.127	-0.274*	0.325**	-0.094
In December (N = 51)												
Adults												
Coleoptera												
Elmidae												
<i>Stenelmis nipponica</i>	0.5	1.3	0	0	8	24	-0.335*	0.203	0.087	-0.093	0.071	n.a.
<i>Zaitavia awana</i>	0.5	1.1	0	0	5	24	-0.228	0.243ms	0.095	-0.096	0.068	n.a.
<i>Zaitaviaria brevis</i>	0.6	1.0	0	0	3	32	-0.292*	0.079	0.145	0.081	0.105	n.a.
ms P<0.1, * P<0.05, ** P<0.01, *** P<0.001												

ms P<0.1, \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

significant in Psephenid *Mataeopsephus japonicus* (Matsumura, 1916) in August. Most of the correlations with water depth were negative and showed significance in some species, except for the significant positive relationship in stonefly *Neoperla* sp. The number of *Z. awana* adults, and mayfly *Baetis* sp., stonefly *Neoperla* sp., caddisfly *Hydropsyche orientalis* Martynov, 1934, caddisfly *Macrostemum radiatum* (McLachlan, 1872), and caddisfly *Gumaga* sp. larvae were positively correlated with current velocity. The number of individual mayfly species of *Ecdyonurus yoshidae* Takahashi, 1924 and *Choroterpes altioculus*

214 Kluge, 1984 was negatively correlated with current velocity. Figures 5 (August) and 6 (December) provide  
 215 diagrams that show the relationship between the number of elmid beetles and environmental factors.



Best subsets multiple regressions

Table 5 shows the standardized partial regression coefficients for the selected environmental variables for insect numbers. *Zaitzevia awana* larvae were excluded from the regression analysis because the total number of individuals was small ( $n < 10$ ).

In August, the negative effects of PC1 (preferring large pebbles) and water depth (preferring shallowness) were significant for *S. nipponica* and *Z. brevis* adults, and *S. nipponica* larvae. The positive effect of PC2 (preferring small pebbles and evading very large pebbles) was significant for *Z. brevis* larvae. For adult *Z. awana*, the positive effects of current velocity and distance from the closest terrestrial area were significant, and PC3 was marginally affected.

For the larvae of the two psephenid species (*Eubrianax*), the negative effects of PC1 and water depth were significant. Another psephenid species (*M. japonicus*) was negatively affected by PC1 and positively affected by PC3 and distance from the closest terrestrial area.

Table 5 Standardized partial regression coefficients of environmental variables in best subsets regressions.

Month / Stage / Species	Substrate			Water depth	Current velocity	Distance from terrestrial area	Adjusted R <sup>2</sup>	Probability
	PC1	PC2	PC3					
August (N = 64)								
Adults								
<i>Stenelmis nipponica</i>	-0.308*			-0.274*	0.185		0.160	0.004
<i>Zaitzevia awana</i>	-0.187		0.226ms		0.317*	0.270*	0.131	0.015
<i>Zaitzeviaria brevis</i>	-0.298*			-0.271*			0.137	0.040
Larvae								
<i>Stenelmis nipponica</i>	-0.333**			-0.347**	0.220ms		0.232	<0.001
<i>Zaitzeviaria brevis</i>		0.319**		-0.229ms			0.164	0.002
<i>Eubrianax granicollis</i>	-0.285*			-0.354**			0.184	0.001
<i>Eubrianax ranicornis</i>	-0.461***	0.170	0.176ms	-0.257*			0.324	<0.001
<i>Mateopsephus japonicus</i>	-0.295*	-0.204ms	0.349**		0.235ms	0.334**	0.215	0.002
<i>Baetis</i> spp.		-0.234ms		-0.437**	0.454***		0.218	<0.001
<i>Ecdyonurus yoshidaei</i>	-0.386**			-0.179	-0.264*		0.205	0.001
<i>Choroterpes alticola</i>	-0.267*			-0.461***	-0.227*	-0.180	0.323	<0.001
<i>Ephoron shigae</i>	-0.408**				-0.263*		0.173	0.001
<i>Neoperla</i> sp.					0.546***		0.287	<0.001
<i>Hydropsyche orientalis</i>	-0.180ms			-0.287**	0.575***		0.366	<0.001
<i>Macrostemum radiatum</i>					0.565***	0.205ms	0.268	<0.001
<i>Gumaga</i> spp.	-0.492***			-0.352**	0.334**		0.451	<0.001
December (N = 51)								
Adults								
<i>Stenelmis nipponica</i>	-0.335*	0.203					0.118	0.018
<i>Zaitzevia awana</i>	-0.228	0.243ms					0.074	0.059
<i>Zaitzeviaria brevis</i>	-0.292*						0.066	0.038
ms P<0.1, * P<0.05, ** P<0.01, *** P<0.001								

ms  $P < 0.1$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

Non-coleopteran larvae exhibited contrasting current velocity patterns. *Baetis* spp. and *H. orientalis* were negatively affected by water depth and significantly positively affected by current velocity. The



number of *Neoperla* sp. and *M. radiatum* individuals also showed a significant positive relationship with current velocity. *Gumaga* sp. was negatively affected by PC1 and water depth and had a significant positive relationship with current velocity. *Ecdyonurus yoshidae* and *Ephoron shigae* (Takahashi, 1924) were significantly negatively affected by PC1 and current velocity. *Choroterpes altioculus* was significantly negatively affected by PC1, water depth, and current velocity.

In December, only the substrate variables were selected in the regression analyses for the adult elmids. The negative effect of PC1 was significant in *S. nipponica* and *Z. brevis*. In *Z. awana*, PC2 (virtually corresponding to PC3 in August) had a marginally positive effect.

# Structure of species assemblages in August

Three PCA axes explained 56 % of the total variance in species assemblages in August (Fig. 7). All the species bore positive scores on the first axis, which explained 32.0 % of the total variance. Adults of elm mid species (Fig. 7: 1–3) were located in a highly positive position on the first axis and in the middle on the second and third axes, which differed from other aquatic insect habitat preferences. The larvae of elmids (Fig. 7: 4, 5) were clearly separated from adults and were located in the middle on the first and second axes, and in a low negative position on the third axis.

The first axis was negatively correlated with substrate PC1 and water depth, and positively correlated with current velocity. The second axis was negatively

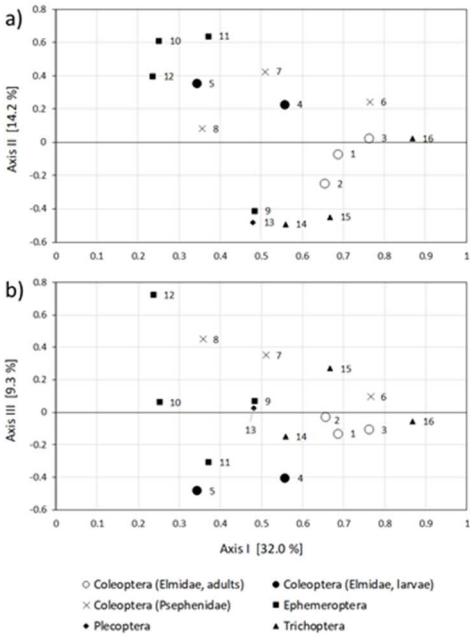


Table 6 Pearson's correlation coefficients between PCA axes of species assemblages and environmental variables (N = 64).

PCA axis of assemblages	Substrate			Water depth	Current velocity	Distance from terrestrial area
	PC1	PC2	PC3			
I	-0.507***	0.241	0.168	-0.331**	0.355**	0.056
II	-0.237ms	-0.008	-0.134	-0.336**	-0.530***	0.047
III	-0.109	-0.335**	0.030	0.232ms	-0.053	0.175

ms P<0.1, \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

correlated with water depth and current velocity. The third axis was negatively correlated with substrate PC2 (Table 6).

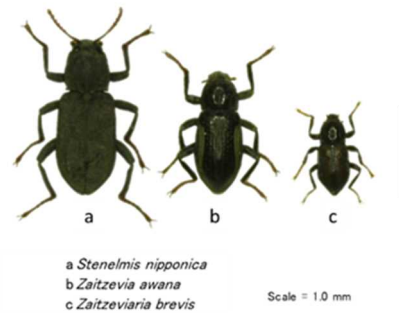
## Discussion

Substrate condition is one of the most important benthic environmental factors that affects the inhabitation of aquatic insects (Yoshimura 2012). The number of individuals of all aquatic insect species was negatively correlated with PC1, which was significant in many species. This indicates that most species examined in this study were abundant where the substrate comprised larger pebbles (>31.5 mm) and less granules and sand (< 4 mm).

The pebble substrate provides an interstitial habitat space, a foothold for insects to cling to, space for water flow, and a surface on which algae and mosses grow. Some elmids generally inhabit coarse-grained substrates (Malmqvist and Sjöström 1983). An European species, *Elmis maugetii*, is abundant on coarse substrates with high water velocity, whereas another species, *Riolus subviolaceus*, is abundant in small grain size sediments with high water velocity (Dietrich and Waringer 1999). Substrate characteristics are the major determinants of the presence of North American *Optioservus sandersoni* and *Stenelmis lateralis* larvae (Lloyd and Sites 2000). Our study revealed that the adults and larvae of the examined elmid species tended to inhabit larger pebbles and fewer sand substrates. Additionally, species-specific and stage-specific preferences were observed. The adult elmid beetles *S. nipponica* (Fig. 5a) and *Z. brevis* (Fig. 5c) preferred substrates with more pebbles (~ 31.5 mm) and less granules and sand (~ 4 mm). *Zaitzevia awana* (Fig. 5b) did not appear to prefer a small pebble (4–8 mm) substrate in August. *Zaitzeviaria brevis* larvae (Fig. 5c) preferred medium-sized pebbles (8–16 mm) over large pebbles (~ 31.5 mm). *Stenelmis nipponica* larvae (Fig. 5a) preferred substrates with large pebbles and fewer granules and sand (~ 4 mm) in August. In December, adults also showed a preference for large pebbles with fewer granules and sand in *S. nipponica* (Fig. 6) and *Z. brevis* (Fig. 6c).

According to Tominaga et al. (2012), *Z. awana* and *Z. brevis* inhabit small gravels in riffles, whereas

281 *S. nipponica* is found in riffles with a large water volume and plant bodies. Although we could not find any  
 282 obvious plants in the river, our results showed a similar tendency that *S. nipponica* prefer large pebbles with  
 283 fewer granules and sand. This means that this species prefers a larger substrate size for clinging to. The  
 284 body length of adult *S. nipponica*, *Z. awana*, and *Z. brevis* is approximately 3.0 mm, 2.0 mm and 1.5 mm,  
 285 respectively (Sato 1985; Fig. 8). The difference in body  
 286 size results in interspecies differences in preferred  
 287 substrate conditions. The significant negative effects of  
 288 PC1 on *S. nipponica* larvae and the significant positive  
 289 effect of PC2 on *Z. brevis* larvae might also be due to the  
 290 difference in body size between them.



291 A significant negative effect of water depth (preferring shallowness) was detected in *S. nipponica* and  
 292 *Z. brevis* adults and *S. nipponica* larvae, and a marginal effect was detected in *Z. brevis* larvae. A preference  
 293 for shallow depths of less than 30 cm is found in *Limnius letourneuxi*, *Grouvellinus caucasicus*, and *Elmis*  
 294 *rioloides* (Degani et al. 1993). Generally, the closer to the water surface, the greater the amount of dissolved  
 295 oxygen. Therefore, proximity to the water surface will help insects gain more oxygen. Proximity to the  
 296 water surface might also partly help larva reach the river margin to pupate. The algal growth on stones,  
 297 which tends to grow in the shallows, also contributes to an increase in the amount of dissolved oxygen near  
 298 the surface. Algae and moss, as well as benthic particle size and current velocity, are crucial for the  
 299 abundance of some elmids (Malmqvist and Sjöström 1983; Dietrich and Waringer 1999).

300 Although adult elmids require running water for plastron respiration (Thorpe 1950), tolerance to current  
 301 velocity seems to differ among species (Degani et al. 1993). Although elmid beetles pupate out of the water  
 302 (Elliot 2008), the distance from the closest terrestrial area did not seem to have a large effect on the elmid  
 303 beetles observed in this study. There may be a preferred substrate type for pupation, not to pupate on the  
 304 nearest land. The significant positive effects of current velocity and distance from the closest terrestrial area  
 305 and marginal positive effects of PC3 were only observed for adult *Z. awana*, indicating different



environmental conditions are required by the other two elmids species. Despite the sandy substrate preference of this species, a rapid stream is more important than substrate condition. Further, the preference of substrate condition might be partly due to the relationship between the distance from the closest terrestrial area and current velocity.

A psephenid species, *M. japonicus*, was negatively affected by PC1 and positively affected by PC3 and distance from the closest terrestrial area, which was similar to the preference for substrate conditions in adult *Z. awana*. Generally, *M. japonicus* inhabits sand substrates rather than gravel substrates (Kawai and Tanida 2005), which might lead to a relationship with PC3. Correlation with the distance from the closest terrestrial area and the marginal relationship with current velocity, but no relationship with water depth, indicates that they get oxygen through water flow, not through close proximity to surface water. In contrast, *Eubrianax* spp. showed a different pattern to that of *M. japonicus*, preferring shallow water. This indicates that their route for obtaining oxygen is close to the river surface. The distribution of *Psephenus herricki* is related to river turbulence, the geographic distribution of riffles, and current velocity (Murvosh 1971; Lloyd and Sites 2000). The environmental conditions required for their inhabitation largely differed among species, even within the same family.

Current velocity had a significantly greater effect on non-coleopteran aquatic insects than on the five beetle species. *Baetis* spp. can move frequently among pools and riffles (Kawai and Tanida 2005); thus, the results of this study show that they prefer rapid current velocities in shallow areas. By contrast, three other mayflies in this study preferred slow current velocity with a coarse substrate. Generally, they inhabit the middle to lower reaches of the river. *Choroterpes altiocularis* is a collector that is found under the stones in moderately shallow pools; *E. yoshidae* is found in riffles with slow current velocity; and *E. shigae* is a burrower that inhabits erosional and depositional lotic areas (Kawai and Tanida 2005). Preference for rapid current velocity by *Neoperla* sp. was also consistent with the general knowledge of congener behavior (Kawai and Tanida 2005).

*Macrostemum radiatum* showed a preference for rapid current velocity, although water depth was

not a factor for its existence. Furthermore, the distance from the closest terrestrial area was marginally explained. By contrast, in *H. orientalis*, water depth was negatively explained and current velocity was positively explained. The larvae of *M. radiatum* construct larger mesh nets than those of *H. orientalis* (Kawai and Tanida 2005), which likely leads to differences in habitat preference. The size of the larvae might also be indirectly related to differences in their habitat preferences.

Habitat preference differed among the aquatic insects in this study. In addition, the results also clarified the differences among species and stages of beetles. Interspecific differences in habitat preference were large among the larvae of elmids and psephenid beetles, whereas others were small among the adult elmid beetles. Further, the environmental factors required for suitable habitat conditions differed between adult and larval elmid beetles. These results indicate there is a need for scrupulous attention to the conservation of rare species.

The coefficients of variation of each river substrate size class were larger in August than in December, indicating there were more frequent substrate disturbances in summer. Only substrate variables were selected for the abundance of adult elmids. Fewer factors explaining the number of elmid beetles in December would be caused by less benthic disturbance in winter.

As moderate substrate disturbance results in a higher diversity of aquatic insects (e.g., Collier and Quinn 2003; Yoshimura 2012), substrate conditions are the main factor determining the inhabitation of elmid beetles. In addition, higher quality flows and physically well-constructed habitats are needed in order to conserve the elmid species in spring outlets (Bowles et al. 2003; Cooke et al. 2015). Riparian buffer vegetation is important for elmid beetle assemblages because it prevents polluted water from terrestrial areas from flowing directly into the stream (Braun et al. 2018). For the conservation of endangered elmid species in Japan (Ogata and Nakajima 2006), the required substrate structure, and vegetation and water conditions of these species should be investigated further.

We have also found that the environmental factors required for suitable habitat conditions differed between the adult and larval stages of elmid beetles, as well as among species. Although elmid beetles

require highly sensitive habitats, they are easily surveyed (e.g., McCreddie and Bedwell 2014). Therefore, they are convenient indicators of riverbed environments. To conserve rare elmids species, meticulous research should be conducted, which would also be helpful for the conservation of other aquatic insects.

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## Figure Legends

**Fig. 1** Location of the study site on the Mukogawa River, Hyogo Prefecture, Japan.

**Fig. 2** An aerial photo of the research area (Google Maps 2021). The white arrow indicates the location of the study site.

**Fig. 3** Detailed maps of the study site and the largest percentage of substrata class size in a) August and b) December. Rhombus size indicate the substrate size class account for the greatest proportion of riverbed substrate.

**Fig. 4** Detailed maps of the study site in a) August and b) December. Each grid is 2 m × 2 m. One quadrat was set in each square of the grids. Water depth, current velocity, and substrate type in each quadrat (25 cm × 25 cm) are indicated within each square of the grids. Dark orange: permanent terrestrial area. Light orange: temporal terrestrial area. The size of the arrow indicates water depth and current velocity. Width of the arrow: water depth. Length of the arrow: current velocity. The pattern and color of the arrows indicate substrate type. Patterned arrow: PC1. Colored arrow: PC2.

**Fig. 5** Detailed maps of the study site and abundance of elmids in August. a) *Stenelmis nipponica*, b) *Zaitzevia awana*, c) *Zaitzeviaria brevis*. Each grid is 2 m × 2 m. One quadrat was set in each square of the grids. Water depth, current velocity, and substrate type in each quadrat (25 cm × 25 cm) are indicated within each square of the grids. Solid line circles indicate the number of adult elmids sampled. Dotted line circles indicate the number of larval elmids sampled. Dark orange: permanent terrestrial area. Light orange: temporal terrestrial area. The size of the arrow indicates water depth and current velocity. Width of the arrow: water depth. Length of the arrow: current velocity. The pattern and color of the arrows indicate substrate type. Patterned arrow: PC1. Colored arrow: PC2.

**Fig. 6** Detailed maps of the study site and abundance of elmids in December. a) *Stenelmis nipponica*, b) *Zaitzeviaria brevis*. Each grid is 2 m × 2 m. One quadrat was set in each square of the grids. Water depth, current velocity, and substrate type in each quadrat (25 cm × 25 cm) are indicated within each square of the grids. Solid line circles indicate the number of adult elmids sampled. Dark orange: permanent terrestrial area. Light orange: temporal terrestrial area. The size of the arrow indicates water depth and current velocity. Width of the arrow: water depth. Length of the arrow: current velocity. The pattern and color of the arrow indicate substrate type. Patterned arrow: PC1. Colored arrow: PC2.

**Fig. 7** Principal components analysis (PCA) scatterplots of species assemblages collected in August. a) axes I and II, and b) axes I and III. Values in brackets indicate the proportion of variance explained. 1–3, adults; 4–16, larvae. 1, 4, *Stenelmis nipponica*; 2, *Zaitzevia awana*; 3, 5, *Zaitzeviaria brevis*; 6, *Eubrianax granicollis*; 7, *Eubrianax ramicornis*; 8, *Mataeopsephus japonicas*; 9, *Baetis* spp.; 10, *Ecdyonurus yoshidae*; 11, *Choroterpes altioculus*; 12, *Ephoron shigae*; 13, *Neoperla* sp.; 14, *Hydropsyche orientalis*; 15, *Macrostemum radiatum*; 16, *Gumaga* sp.

**Fig. 8** Adults of three elmids species, habitus, dorsal view: a) *Stenelmis nipponica*; b) *Zaitzevia awana*; c) *Zaitzeviaria brevis*. Scale = 1.0 mm.