CSIRO PUBLISHING

Marine and Freshwater Research, 2019, **70**, 504–512
https://doi.org/10.1071/MF18031

Assessing alpha and beta diversities of benthic macroinvertebrates and their environmental drivers between watersheds with different levels of habitat transformation in Japan

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Abstract. Little is known about differences in species diversity among ecological communities subject to different levels of human-caused habitat transformation and how this disturbance contributes to diversity through symbiotic dependencies with the environment in freshwater ecosystems. We estimated α and β diversities of benthic macroinvertebrates and relationships between diversity and environmental variables in Ado River (natural) and Yasu River (intermediately disturbed) watersheds, Japan. Alpha diversity was consistently slightly higher in the natural river watershed than in the intermediately disturbed one, but the spatial distribution was not equivalent. The opposite pattern was found for β diversity. Significant differences in environmental variables existed between the two river watersheds, with especially high chlorophyll-*a* concentrations detected in the intermediately disturbed watershed. Alpha diversity was not correlated with specific environmental variables, whereas water temperature and chlorophyll-*a* concentrations were the two most significant environmental variables influencing β diversity across sites in the two watersheds. These results suggest that diversity patterns in freshwater benthic macroinvertebrates are differentially influenced by levels of human-caused habitat transformation, especially that intermediately disturbed habitats may benefit species turnover, and further understanding how they relate to environmental variables is essential for protecting local to regional diversity and can provide useful information for conservation planning to maximise biodiversity at the watershed scale.

Received 24 January 2018, accepted 10 September 2018, published online 21 November 2018

Introduction

Knowledge of the effects of habitat transformation on species distribution and abundance is important to biodiversity conservation. Human-caused habitat transformation can affect wildlife by directly changing habitat quality and restricting resources, which, consequently, influence species distribution, behaviour, demography, population size and diversity (Gill 2007). Notably, recent studies have shown that biodiversity can benefit from moderate anthropogenic disturbance because of increases in habitat heterogeneity and the associated decreases in competitive interactions, which prevent competitive exclusion in intermediately disturbed habitats (Hamer and Hill 2000; McCabe and Gotelli 2000; Kessler 2001; Svensson *et al.* 2012). Therefore, understanding how species abundance or diversity vary spatially in response to human-caused habitat transformation is of paramount value in the development of suitable mitigation measures and conservation plans during the current age of rapid global environmental changes (Socolar *et al.* 2016).

Exploration of the factors that influence the spatiotemporal dimensions of local diversity, community assemblage compositions and ecosystem functions has been an enduring theme in ecology and conservation biology (Huston 1994; Gaston 2000; Witman *et al.* 2004), and such local-scale ecological effects are important because local communities are integral components of larger biogeographic regions, influencing the composition of larger-scale species pools, for example, regional diversity, as the ultimate determinant of local species richness (Ricklefs 1989; Witman *et al.* 2004; Wilson 2008; Burley *et al.* 2016). In addition, studies have shown that different diversity measurements may not necessarily exhibit the same patterns in response to changing environmental conditions (Witman *et al.* 2004; Wilson 2008), which is relevant to both α diversity, described by the number of taxa and their abundance within communities or habitats (typically measured at the site scale), and β diversity, defined as the variation in community composition and measured in terms of pair-wise dissimilarity among sites (Whittaker 1972). Thus, multiple assessments of diversity patterns in relation to environmental change are essential because extrapolations from one observation to another are fraught with risks of making incorrect inferences.

Benthic macroinvertebrates play fundamental roles in freshwater ecosystems because they serve as channels through which bottom-up and top-down forces are transmitted in food webs. They also functionally influence energy flows and nutrient cycling (Covich *et al.* 1999; Wallace *et al.* 1999). Moreover, the characterisation of benthic macroinvertebrate diversity and assemblages is commonly used in bioassessments of water-quality conditions (Lenat 1988; Plafkin *et al.* 1989; Fore *et al.* 1996). In lotic ecosystems, physical and chemical variables, for example, water temperature, water depth, water-current velocity, pH, dissolved oxygen (DO), have been shown to influence the distribution, abundance and community composition structure of benthic macroinvertebrate species (Rousi *et al.* 2011; Rousi *et al.* 2013; Krepski *et al.* 2014). For instance, high diversity and abundance of benthic macroinvertebrates were characterised by low water temperature but high DO in north-western Poland as well as northern Baltic Sea. Water depth affected zonation of the benthic macroinvertebrate species whereas pH showed the great correlation with the density of benthic macroinvertebrates. Besides, the properties of river networks also strongly influence benthic species assemblages and zonation (Rousi *et al.* 2011; Krepski *et al.* 2014). With the growing human population and urbanisation resulting in regional habitat transformation and degradation, the associated impacts of environmental variables on benthic macroinvertebrate diversity are of concern.

Lake Biwa as the third-oldest lake in the world and the largest freshwater lake in Japan (surface area = 670.3 km^2, maximum depth = 103.6 m, average depth = 41.2 m), and impacts on its water regime and aquatic species communities have been observed as a result of environmental changes (Okuda *et al.* 2012). The upper-river watersheds of Lake Biwa, which determine the water quality and species origins of the lake, have different development scenarios representing natural to disturbed habitats and, thus, provide an opportunity to study the biodiversity dynamics of aquatic biota and their ecological consequences between and within watersheds. In the present study, we explored both the α and β diversity of benthic macroinvertebrate communities in two river watersheds of Lake Biwa with different levels of human-caused habitat transformation, i.e. natural in the Ado River watershed and intermediately disturbed in the Yasu River watershed. We hypothesised high biodiversity in the Yasu River watershed because disturbance may enhance habitat heterogeneity and contributions of specific environmental variables. The primary aims of this study were (1) estimation of differences in α and β diversity between the watersheds, and (2) establishment of predictive relationships between diversity and a subset of environmental variables within each watershed, using multiple regression analysis.

Materials and methods

Study area

The Ado River and Yasu River are the two major tributaries to Lake Biwa (Fig. 1), and the degree of transformation of physicochemical habitat characteristics owing to land development differs significantly between the two catchment areas of the watersheds: forest, agricultural land and urban areas cover 91.5, 3.7 and 0.7% of the former catchment area respectively and 57.7, 22.5 and 6.5% of the latter. Thus, we here considered the Ado River watershed as a natural (i.e. less disturbed) system in this study, and the Yasu River watershed was classified as having an intermediate degree of human-caused habitat transformation.

Biotic and physicochemical environmental data

In both the Yasu River and Ado River watersheds, synoptic surveys were conducted in October in 2012 and 2014 respectively, when flooding is minimal during the productive season. Considering the spatial variation in land-use patterns and stream order, 30 sampling sites were established in each watershed, and benthic macroinvertebrates were collected in duplicate from the streambeds of riffles using a Surber sampler (30 × 30 cm, 475-μm mesh, Rigo, Tokyo, Japan). To ensure sufficiently replicated benthic macroinvertebrate samples for individual land-use types, three land-use types were represented by an equal number of local sites in the Yasu River, whereas at least two local sites representing one land-use type were selected because of the limited agricultural and urban development within the Ado River watershed. The benthic macroinvertebrates were sorted,

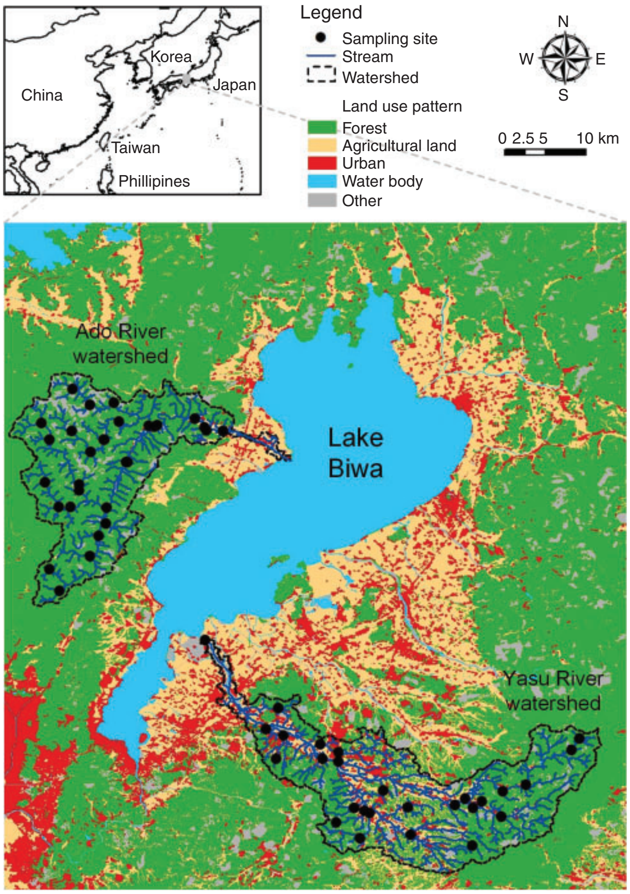

Fig. 1. Map of the study area, showing the land-use patterns and sampling sites for the Ado River and Yasu River watersheds, Japan. For the land-use pattern, 'agricultural land' includes orchard, rice paddy and farmland and 'other' includes pasture, bamboo, grasslands, clearcutting sites, natural outcrops, mining sites, for example.

identified to the genus and species levels, and counted in the laboratory. Data were excluded from one local site in the Yasu River watershed because the riverbeds were disturbed by flooding just before sampling.

Five cobbles were collected to estimate epilithic biomass (i.e. chlorophyll-*a* concentration) as an indicator of primary productivity at each local site. The epilithon was first scraped from a 6×6-cm surface area of individual cobbles with a toothbrush and then filtered through a 150-µm mesh net to remove benthic animals and coarse particulate organic matter. In the laboratory, chlorophyll-*a* was extracted from the epilithic samples in a 90% acetone solution, and its concentration was measured following Scientific Committee on Oceanic Research (SCOR)–United Nations Educational, Scientific and Cultural Organization (UNESCO) spectrophotometric procedures (SCOR-UNESCO 1966) with a Shimadzu UV-1700 spectrophotometer (Kyoto, Japan).

We estimated 10 environmental variables at each local site, including stream order, confluence link (C-link), river depth (cm), river width (m), river discharge ($m^3\ s^{-1}$), water current velocity (cm s^{-1}), DO (mg $O_2\ L^{-1}$), pH, water temperature (K) and canopy openness. Stream order and C-link were obtained from a digitised stream-network dataset based on a 50-m grid digital elevation model (DEM). The stream order was generated from the hierarchical position of a site in the drainage network and it increased with the confluence of two equally ordered streams (Strahler 1957). The C-link was the number of confluences downstream along a direct path to the mouth of the main channel (Fairchild *et al.* 1998); sampling sites in the upper reaches of a watershed typically have larger C-link values. To measure river depth, river width, river discharge and river water-current velocity, we established five lateral transects at intervals of approximately the same length as the wetted width of the reach and measured the wetted width at each transect to obtain the mean wetted width of each study reach. River depth was measured at 5–10 equally spaced points along each transect to obtain the mean depth of each study reach. The river discharge was measured along a transect with a uniform cross-section by using a current meter (CR-7 WP, Cosumo Riken, Inc., Osaka, Japan). The velocity of the river current of each study reach was estimated by dividing the discharge by the product of the river width and the river depth in that reach, and DO and pH were measured using multiprobes (U-22, Horiba, Kyoto, Japan, and YSI, 556 MPS, YSI Inc., Yellow Springs, OH, USA). The water temperature was calculated as the daily average of 3 weeks of monitoring by using a water temperature logger (Thermochron G, KN Laboratories Inc., Osaka, Japan). The canopy openness was evaluated as the ratio of the daily integrated photon-flux density value (µmol $m^{-2}\ s^{-1}$) monitored using a PAR logger (UIZ-PAR-LR, UIZIN, Tokyo, Japan) to the theoretical PAR values calculated by the FITSOLAR model (Fee 1990). Considering the multicollinearity among some environmental variables, which were evaluated by univariate analysis, and the biological relevance of individual variables to river ecosystems, six variables and the chlorophyll-*a* concentration were finally selected for further analyses (see Tables S1 and S2, available as Supplementary material to this paper).

Diversity analyses

We assessed the diversity of benthic macroinvertebrate communities on the basis of the species richness and abundance for each local site, and examined the nearness or similarity of these parameters among sites within each watershed (McKenna 2003). Four common indices were calculated to assess both site-scale α (richness and Shannon H') and β (i.e. pair-wise dissimilarity in community composition, Bray–Curtis and Sørensen dissimilarities) diversity. Richness is the number of different species at each site on the basis of presence–absence data (Patil and Taillie 1982); species found in any of the duplicate samples at each site were regarded as being present. The Shannon H' index measures both the number of species and the relative abundance of different species in a community (Patil and Taillie 1982); the abundance of individual species at each site was the average of the duplicates. For the β diversity dissimilarity coefficients, Bray–Curtis dissimilarity was determined for the abundance data, and the Sørensen coefficient was used for the presence–absence data. The Sørensen coefficient is 'a broad-sense' measure because it incorporates both species richness and compositional differences among sites (Koleff *et al.* 2003; Podani and Schmera 2011).

The Wilcoxon signed-rank test was used to determine significant differences in the α and β diversities of benthic

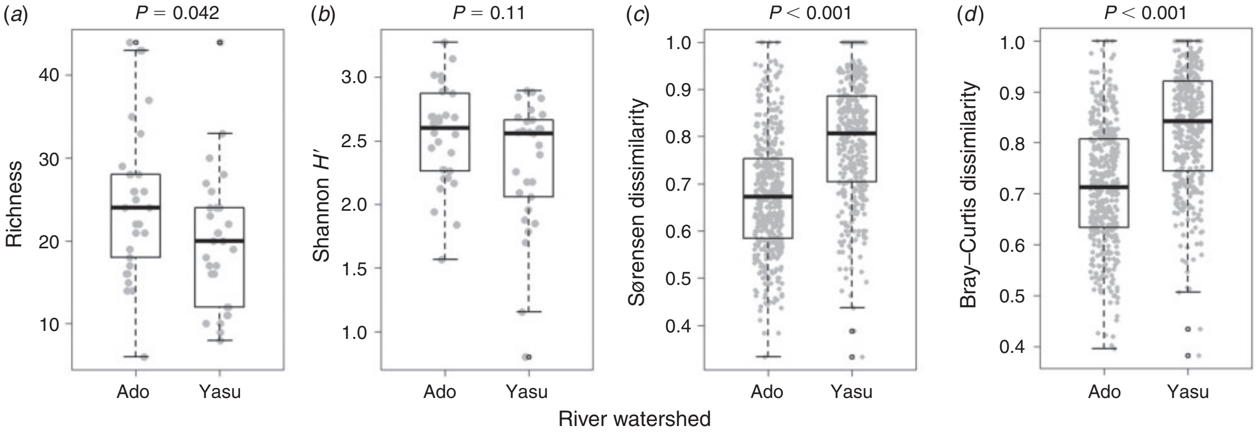

Fig. 2. Box plots of benthic macroinvertebrate diversity indices for the Ado River and Yasu River watersheds, Japan: (*a*) richness, (*b*) Shannon H', (*c*) Sørensen dissimilarity, and (*d*) Bray–Curtis dissimilarity. Differences (*P* values) in benthic macroinvertebrate compositions between the two river watersheds are shown according to the Wilcoxon signed-rank test. The grey circles are individual values across sampling sites; the boxes represent the 25th and 75th percentiles; and the thick horizontal lines inside the boxes are the medians.

macroinvertebrates between the two river watersheds. To further consider the effects of the environmental variables on benthic macroinvertebrate diversity, we fitted multiple regression models using forward stepwise selection of the explanatory variables. Akaike's information criterion (AIC) was used for model selection, with the models with the fewest variables and lowest AIC being selected as the best-fit models. All analyses were performed in R (ver. 3.0.2, R Foundation for Statistical Computing, Vienna, Austria, see https://www.R-project.org/) using the vegan (see https://cran.r-project.org/web/packages/vegan/index.html), Hmisc (see https://cran.r-project.org/web/packages/Hmisc/index.html) and phytools (see https://cran.r-project.org/web/packages/phytools/index.html) packages.

Results

The two river watersheds with different levels of human-caused habitat transformation showed diverse benthic macroinvertebrate communities (Table S3). In the Ado River watershed, the most abundant (~15–16%) benthic macroinvertebrate families were Ephemerellidae and Baetidae, which varied in relative abundance from 0 to 48.5% and from 0.7 to 51.6% across sites respectively. In the Yasu River watershed, by contrast, Naididae and Chironomidae were the two most dominant families, with relative abundances ranging from 30 to 32%.

Alpha diversity (richness and Shannon H') was consistently slightly higher in the Ado River watershed than in the Yasu River watershed (Fig. 2*a*, *b*). Across sites, richness ranged from 6 to 44 and from 8 to 44 species, with the maximum number of benthic macroinvertebrate individuals ranging from 9 to 136 and from 6 to 1377 in the Ado River and Yasu River watersheds respectively. The opposite pattern was found for β diversity, with both Sørensen and Bray–Curtis dissimilarities being significantly higher in the Yasu River watershed than in the Ado River watershed (for both, $P < 0.001$; Fig. 2*c*, *d*). The results implied that the among-site variation in species assemblage composition within each watershed was higher in the intermediately disturbed environment than in the natural one.

There were no consistent spatial patterns in species richness or Shannon H' diversity, especially in the Yasu River watershed, but such α diversity indices were fairly high at the mainstream sites of the Ado River watershed (Fig. 3).

Significant differences were found in the C-link, water current velocity, water temperature and chlorophyll-*a* concentration between the Ado River and Yasu River watersheds (Table 1). The higher chlorophyll-*a* concentration measured in the Yasu River watershed implied that more nutrients were being introduced by humans, resulting in algae blooms throughout the river. Different environmental variables correlated with the α diversity of benthic macroinvertebrates between the Ado River and Yasu River watersheds (Table 2), but the variables were more consistently related to β diversity in both watersheds (Table 3). Moreover, the models generally exhibited a better fit in the Ado River watershed than in the Yasu River watershed, indicating that environmental variables better predicted site-diversity trends in the natural habitats.

Among the environmental variables, the α diversity of the Ado River watershed was best explained by water temperature, chlorophyll-*a* concentration, water current velocity, and pH in the multiple regression models (richness: AIC 218.24, $R^2 = 0.313$, $P = 0.045$; Shannon H': AIC 22.20, $R^2 = 0.430$, $P = 0.002$, Table 2). However, the α diversity of the Yasu River watershed was best modelled by different environmental variables, including C-link, pH, river depth, and chlorophyll-*a* concentration (richness: AIC 173.95, $R^2 = 0.230$, $P = 0.129$; Shannon H': AIC 14.06, $R^2 = 0.753$, $P < 0.001$; Table 2). In contrast to the lack of consistent relationships between α diversity and the environmental variables between the watersheds, water temperature and chlorophyll-*a* concentration were the two most significant variables influencing β diversity (Sørensen and Bray–Curtis dissimilarities) across sites in both watersheds (Table 3). The best model predicting β diversity significantly explained 51.8 and 49.1%, and 18.1 and 19.6% of the variance in these independent environmental variables (in bold in Table 3) for the Sørensen and Bray–Curtis dissimilarities of the Ado River and Yasu River watersheds respectively (for all, $P = 0.001$).

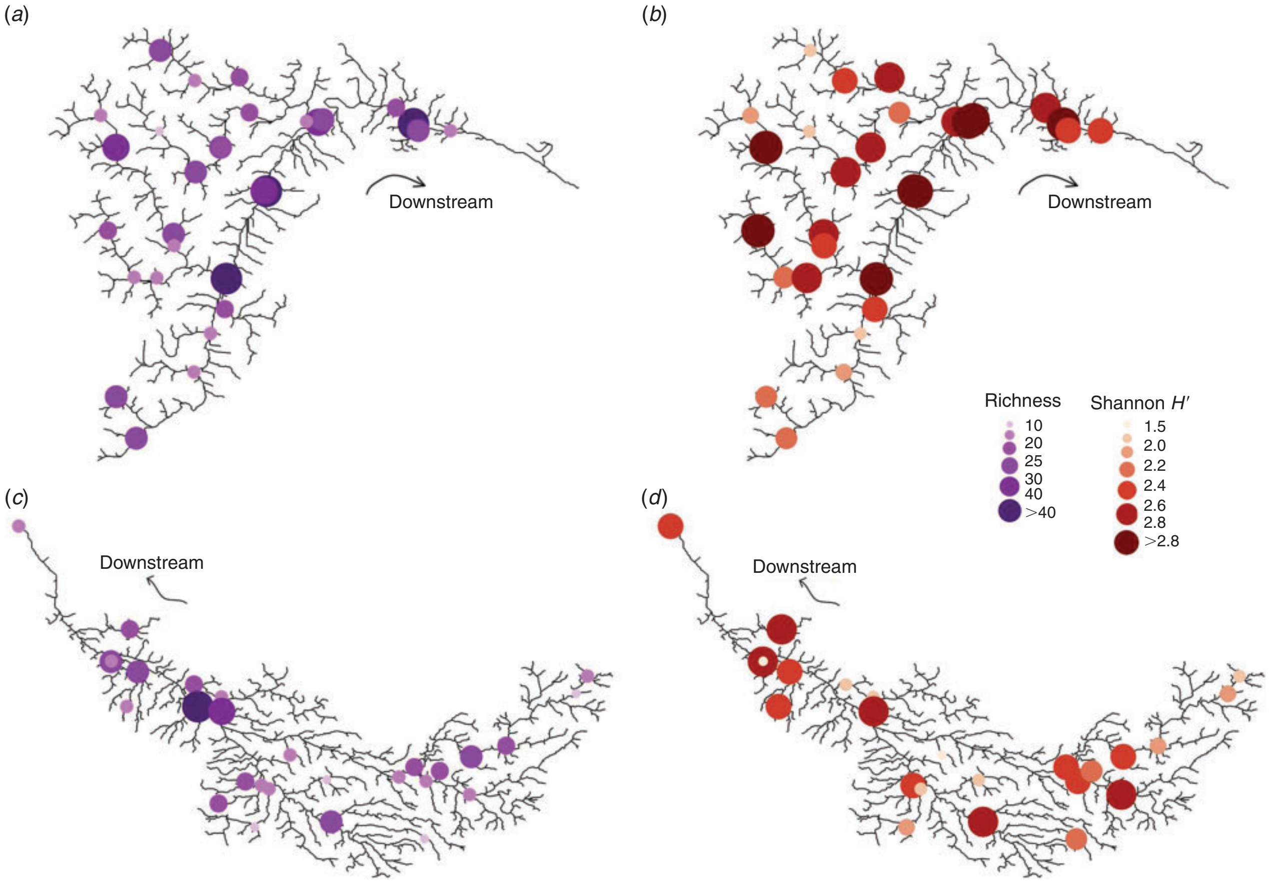

Fig. 3. Species richness in (*a*) the Ado River and (*c*) Yasu River watersheds, and Shannon H' diversity patterns in (*b*) the Ado River and (*d*) Yasu River watersheds. Circles of different colours and sizes show different levels of species richness or Shannon H' diversity.

Table 1. Comparison of environmental variables between the Ado River and Yasu River watersheds, Japan

Variable	Ado River watershed				Yasu River watershed				P (difference)
	Min.	Max.	Mean	s.d.	Min.	Max.	Mean	s.d.	
C-link	5	305	215	108	1	403	324	129	<0.001
River depth (cm)	7.8	62.2	25	14.4	3.6	53.6	20.4	12.5	0.222
Water current velocity ($cm\,s^{-1}$)	10	100.2	49.9	24.6	2.3	55	22.7	13.4	<0.001
Dissolved oxygen ($mg\,O_2\,L^{-1}$)	8.71	10.38	9.61	0.48	8.26	10.81	9.38	0.62	0.143
pH	6.82	7.59	7.28	0.19	6.89	8.3	7.43	0.33	0.256
Water temperature (K)	286.1	289.5	287.7	0.85	286	292.5	290.1	1.36	<0.001
Chlorophyll-*a* ($mg\,m^{-2}$)	0.28	13.15	2.6	2.68	0.29	105	19.07	27.18	0.001

Discussion

The most interesting result of this study was the increase in β diversity in the river watershed with an intermediate level of anthropogenic disturbance compared with that in the natural watershed, supporting the idea that human-caused habitat transformation by land development may increase species heterogeneity, namely, the among-habitat variability in species composition. Because β diversity indices are based on the interaction and combination of all species occurring at two given sites, the increased dissimilarity reflected a higher spatial turnover in community composition within the Yasu River watershed. In fact, except for the variables of river depth and water current velocity, habitat heterogeneity among local sites (i.e. s.d. of environmental variables) was usually higher in the Yasu River watershed than in the Ado River watershed. Moreover, the variations in water temperature and chlorophyll-*a* concentration best explained the Sørensen and Bray–Curtis dissimilarities in both watersheds. These results suggest that the land use in the Yasu River watershed sharpened the environmental gradients in thermal habitats and primary productivity,

Table 2. Pearson's correlation coefficients (*r* and *P* values) and best multiple regression models for benthic macroinvertebrate α diversity and analysed environmental variables in the Ado River and Yasu River watersheds, Japan

Terms in the lowest-AIC multiple regression models for each response group are in bold (with forward selection order in parentheses). Best model R^2 and *P* values are indicated in the final row. n.s., not significant

Variable	Ado River watershed				Yasu River watershed			
	Richness		Shannon *H′*		Richness		Shannon *H′*	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
C-link	0.008	n.s.	−0.007	n.s.	**−0.319 (1)**	n.s.	**0.008 (3)**	n.s.
River depth	0.041	n.s.	0.19	n.s.	**0.253 (3)**	n.s.	**0.197 (2)**	n.s.
Water current velocity	**−0.156 (4)**	n.s.	**0.002 (3)**	n.s.	0.166	n.s.	0.322	n.s.
Dissolved oxygen	0.212	n.s.	0.099	n.s.	−0.024	n.s.	0.439	0.032
pH	**−0.266 (2)**	n.s.	0.096	n.s.	**0.248 (2)**	n.s.	0.457	0.025
Water temperature	**0.325 (1)**	n.s.	**0.454 (1)**	0.012	0.149	n.s.	−0.155	n.s.
Chlorophyll-*a*	**−0.091 (3)**	n.s.	**−0.328 (2)**	n.s.	−0.279	n.s.	**−0.780 (1)**	<0.001
R^2 and *P* of best model	0.313	0.045	0.43	0.002	0.23	0.129	0.753	<0.001

Table 3. Spearman's rho correlation coefficients (ρ and *P* values) and best multiple regression models for benthic macroinvertebrate β diversity and analysed environmental variables in the Ado River and Yasu River watersheds, Japan

Terms in the best multiple regression models for each response group are in bold (with forward selection order in parentheses). Best model ρ^2 and *P* values are indicated in the final row. n.s., not significant

Variable	Ado River watershed				Yasu River watershed			
	Sørensen		Bray–Curtis		Sørensen		Bray–Curtis	
	ρ	*P*	ρ	*P*	ρ	*P*	ρ	*P*
C-link	−0.075	n.s.	−0.08	n.s.	−0.125	n.s.	−0.123	n.s.
River depth	**0.258 (3)**	0.008	**0.308 (3)**	<0.001	−0.026	n.s.	0.004	n.s.
Water current velocity	0.167	0.02	0.176	0.021	**0.129 (3)**	n.s.	0.079	n.s.
Dissolved oxygen	0.109	n.s.	0.089	n.s.	0.086	n.s.	0.111	n.s.
pH	0.083	n.s.	0.065	n.s.	**0.151 (4)**	n.s.	0.084	n.s.
Water temperature	**0.499 (1)**	<0.001	**0.505 (1)**	<0.001	**0.331 (1)**	<0.001	**0.333 (1)**	<0.001
Chlorophyll-*a*	**0.377 (2)**	<0.001	**0.353 (2)**	<0.001	**0.294 (2)**	0.002	**0.320 (2)**	0.002
ρ^2 and *P* of best model	0.518	0.001	0.491	0.001	0.181	0.001	0.196	0.001

thereby increasing the spatial variation in the species composition of the disturbed habitats, and also allowing some species being more common and hence more reliably detected in one river watershed than in the other (e.g. *Drunella* sp., Chloroperlidae gen. sp., *Cincticostella* sp., and *Micrasema hanasense* were the dominant species in the Ado River watershed, and Naididae gen. spp., *Chironomus* sp., *Cheumatopsyche brevilineata* and *Polypedilum* sp. were dominant in the Yasu River watershed), leading to increased β diversity in this study.

Given that connectivity and upstream-downstream position are specific characteristics of rivers (Czapiga *et al.* 2015), we could not clearly interpret spatial distribution in terms of the observed changes in benthic macroinvertebrate α and β diversity in the present study. Our results suggest that the successive substitution of the communities from the upper reaches of the tributaries to the river mouth may depend on factors related to basin relief and corresponds to changes in the local landscape and hydrological conditions rather than the river continuum, which will be discussed in more detail later.

The increase in the abundance of benthic macroinvertebrate species within the assemblages is consistent with our hypotheses, supporting the idea that the species richness of communities is controlled by migration (the available species pool), especially in species with intermediate numbers (i.e. >10 and <100), although the results of such comparisons have shown variable results across different taxa, environments and sampling approaches (McCabe and Gotelli 2000; Stirling and Wilsey 2001; Bock *et al.* 2007; Svensson *et al.* 2012). The variation in the relationships between species presence–absence and abundance in the assemblages was higher in the Yasu River watershed than in the Ado River watershed, not only indicating that little of the variation in the abundance of the assemblages can be explained by species richness but also implying that disturbance caused by habitat transformation can change the balance of forces acting on the local community, increase the strength of interspecific competition to benefit dominant species, and cause different proportions and distributions of each species within the local aquatic community (Stirling and Wilsey 2001; Leveque 2003). Therefore, when focusing on the variability in species composition, biotic interactions (e.g. competition and predation) affecting abundance may play a more important role in governing diversity in intermediately disturbed habitats. Additionally, we found that the dissimilarities in β diversity associated with long distances were higher in the Yasu

River watershed than in the Ado River watershed (data not shown), indicating that habitats with different levels of human-caused transformation and disturbance may differ in species composition and abundance, further leading to increased β diversity (Cramer and Willig 2005).

The environmental variables affecting the α diversity of benthic macroinvertebrate communities differed from the variables affecting β diversity. The differing models among diversity indices were consistent with earlier studies, suggesting that the α and β diversities of benthic macroinvertebrate communities cannot be attributed to any single environmental variable (Karatayev *et al.* 2013). Moreover, the principal environmental variables affecting α diversity in the Ado River and Yasu River watersheds were different, whereas the important variables for β diversity were similar between the watersheds. The results suggest that patterns of diversity should be evaluated at appropriate spatial scales that are hypothesised to regulate α and β diversities (Huston 1999). An additional interesting finding was that our combinations of diversity indices were mainly unrelated to DO in the watersheds, even though oxygen is needed by aquatic organisms for aerobic respiration. This may due to the balance between nutrient enrichment and microbial metabolism in the water column and sediments among the sampled sites in both watersheds that formed environments in which biological oxygen consumption equalled the oxygen supply, thus decreasing the effect of DO in this study. In fact, the two watersheds in the study had higher concentration of DO, on average, than that found in other studies, showing DO as the determinant of composition, abundance and production of benthic species and a major cause of the zonation of benthic macroinvertebrates (Likens 2010; Craig *et al.* 2015). Thus, we infer that, below certain concentrations, DO concentration can be a critical environmental variable influencing benthic macroinvertebrate diversity.

Although consistent correlations between α diversity and the environmental variables were not found between the two river watersheds, all the estimated α diversity values were negatively associated with the chlorophyll-*a* concentration. This contrasts with earlier findings that have highlighted algal production as being positively related to the structure and functioning of river ecosystems (Orive *et al.* 2002; Çelik *et al.* 2010; Frainer 2013). It is possible that the nutritional quality and edibility of periphyton (i.e. fatty acid composition) in streams and rivers with a higher algal production, such as urban and agricultural streams, are reduced partly as a result of the taxonomic shift from diatoms to green algae, thereby decreasing the growth and abundance of macroinvertebrate consumers (Hill *et al.* 2011; Cashman *et al.* 2013; Larson *et al.* 2013); however, the underlying mechanism requires further investigation. In addition, the position in the river network (i.e. C-link in the present study) had significant and interacting effects on α diversity and community dissimilarity in the Yasu River watershed, indicating that river connectivity may affect diversity by closely reflecting dispersal, especially in disturbed systems (Altermatt *et al.* 2013). For β diversity, water temperature and chlorophyll-*a* concentration were the strongest environmental variables in both natural and intermediately disturbed river watersheds, as mentioned above, and this pattern has been found in several studies across many geographic areas and elevations (Jacobsen *et al.* 1997; Graça *et al.* 2004; Heino 2009; Angeler and Drakare 2013; Rousi *et al.* 2013; Krepski *et al.* 2014). By extension, future changes in the temperature gradients in river networks associated with climate change and riparian land development might, therefore, be expected to cause the most dramatic biotic responses at both local and regional scales (Fitzpatrick *et al.* 2013).

Regarding the environmental correlates of the patterns of α and β diversities in benthic macroinvertebrate communities, the predictive capacities of the models differed between both watersheds. The model fits were consistently higher for the natural river watershed than for the intermediately disturbed watershed, expect for the Shannon H' in the Yasu River watershed. This suggests that changes in the measured abiotic environmental conditions were more important for changes in benthic macroinvertebrate diversity in the natural than in the intermediately disturbed habitats. However, in addition to the variance explained by the environmental variables in the models, their residual variance or lack of significant correlations suggests that other, unmeasured variables may also be important and may include biological interactions (e.g. predation and competition) and spatial or stochastic processes (e.g. emigration and immigration and flood disturbance). Nevertheless, human-caused habitat transformation leads to changes in community composition and increases the difficulty in making model predictions.

In general, our best models provided evidence to confirm the following: (1) changes in diversity are associated with different combinations of environmental variables; (2) the contributions of these variables vary between communities in the different river watersheds and with the type of diversity measured; and (3) the explanatory power of the models is higher for the natural habitats, such as the Ado River watershed, than for those that are intermediately disturbed, such as, for example, the Yasu River watershed. To accurately estimate the risk of species losses owing to habitat transformation and to design robust protected-area networks for biodiversity conservation, it is important to understand the process of spatial community organisation. Whether α and β diversities increase, decrease or remain unchanged by human-related factors, including agriculture, selective logging, urbanisation, species invasions, overhunting and climate change, depends on the balance among the processes that cause species compositions to become more different (biotic heterogenisation) or more similar (biotic homogenisation) among sites. Although merely maintaining high α or β diversities is not always a desirable conservation outcome, understanding how α and β diversities vary with anthropogenic disturbance and how they relate to the environment is essential for protecting local to regional diversity and can provide useful information for conservation planning to maximise biodiversity at the watershed scale.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of funding

This work was supported by a JSPS Grant-in aid (number 24370010). Additional support for Chia-Ying Ko from the Ministry of Science and Technology, Taipei, Taiwan, under Grant MOST 105-2119-M-002-059-MY2, and from the College of Life Science, National Taiwan University, under Grant 107L2027, is acknowledged.

Acknowledgements

We are grateful to many people involved in conducting field work and analysing samples. We also thank reviewers for providing invaluable comments and suggestions that helped improve the paper. This work was supported by the RIHN Project (D-06-14200119).

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Handling Editor: Nicholas Davidson

Marine and Freshwater Research, 2019, **70**, 504–512
https://doi.org/10.1071/MF18031

Supplementary material

Assessing alpha and beta diversities of benthic macroinvertebrates and their environmental drivers between watersheds with different levels of habitat transformation in Japan

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Table S1. Values of diversities and environment variables used in this study

River watershed	SITE ID	Year	Richness	Shannon *H′*	Chlorophyll-*a*	Stream order	C-link	River depth	River width	River discharge	Water current velocity	DO	pH	Water temperature	Canopy openness
Ado River	1	2014	29	2.201	5.642	2	303	22.56	2.78	0.103	19.57	9.34	6.818	286.18	0.049
Ado River	3	2014	25	2.260	4.185	2	290	8.20	2.92	0.076	34.32	9.18	7.057	286.10	0.125
Ado River	8	2014	18	2.168	0.759	3	283	19.52	7.00	0.543	41.60	9.37	7.29	287.01	0.194
Ado River	9	2014	14	1.839	2.471	4	28	24.76	9.06	1.423	64.60	9.07	7.149	287.35	0.351
Ado River	13	2014	21	2.491	2.342	3	269	35.00	11.70	0.755	29.23	9.33	7.417	287.31	0.034
Ado River	15	2014	22	2.901	0.613	1	300	10.47	2.03	0.054	27.90	9.41	7.328	286.93	0.038
Ado River	17	2014	16	2.207	0.625	3	42	19.48	9.10	0.955	54.14	9.32	7.041	287.31	0.430
Ado River	20	2014	17	2.677	1.529	1	43	7.80	2.02	0.037	25.01	9.31	7.416	287.12	0.175
Ado River	22	2014	16	2.120	0.282	2	22	10.04	6.32	0.365	57.79	9.11	7.422	287.01	0.688
Ado River	23	2014	35	2.890	0.563	3	128	10.84	5.56	0.189	33.54	9.07	7.38	287.18	0.412
Ado River	26	2014	26	2.699	1.923	4	13	25.36	15.24	2.175	59.21	9.34	7.311	287.36	0.069
Ado River	28	2014	15	2.404	0.409	2	303	15.72	5.30	0.104	12.99	9.39	7.495	287.43	0.008
Ado River	30	2014	43	3.275	0.398	5	5	55.00	19.73	4.757	44.78	9.71	7.42	287.62	0.589
Ado River	32	2014	33	2.973	2.601	2	281	12.84	4.46	0.159	33.91	9.97	7.187	287.60	0.032
Ado River	33	2014	43	3.144	0.555	5	293	36.20	33.18	8.051	70.05	9.6	7.227	288.11	0.427
Ado River	35	2014	28	2.686	2.511	2	287	17.56	5.32	0.437	50.24	10.2	7.374	287.38	0.034
Ado River	37	2014	6	1.565	13.147	1	291	18.84	2.58	0.117	26.93	9.54	7.366	287.16	0.013
Ado River	41	2014	21	2.272	0.805	3	144	38.40	14.28	3.340	63.94	10.38	7.421	287.76	0.166
Ado River	42	2014	26	2.639	0.895	2	144	18.36	5.14	0.688	74.79	9.8	7.306	287.53	0.022
Ado River	43	2014	24	2.273	3.452	3	302	36.76	16.12	5.066	86.25	10.31	7.259	287.92	0.609
Ado River	45	2014	26	1.941	3.117	2	272	24.12	5.98	0.719	52.04	10.07	7.161	287.50	0.044
Ado River	47	2014	19	2.445	2.289	2	270	18.20	4.44	0.488	63.64	10.24	7.586	287.50	0.306
Ado River	48	2014	22	2.690	1.082	3	283	32.68	13.14	3.850	94.66	10.22	7.419	287.89	0.104
Ado River	51	2014	18	2.691	1.023	4	282	39.32	24.00	7.658	81.91	10.32	6.929	288.28	0.707
Ado River	52	2014	37	2.875	3.493	1	169	25.92	3.50	0.087	10.01	10.38	7.213	289.31	0.231
Ado River	53	2014	28	3.009	6.008	5	250	39.64	62.38	15.342	68.65	9.22	7.395	289.33	0.686
Ado River	57	2014	24	2.652	1.405	5	267	46.95	32.72	13.228	100.22	9.01	7.486	288.93	0.660
Ado River	59	2014	44	3.018	7.350	2	277	8.44	3.20	0.047	18.08	9.6	6.956	289.55	0.635
Ado River	60	2014	28	2.561	3.309	2	305	10.28	2.22	0.050	30.62	9.79	7.156	288.40	0.591
Ado River	63	2014	14	2.553	3.195	5	305	62.16	34.50	12.832	67.89	8.71	7.523	288.99	0.787
Yasu River	3	2012	17	2.467	22.883	5	1	53.64	29.20	4.881	32.31	9.25	7.129	292.49	0.633
Yasu River	8	2012	30	2.741	2.486	2	402	15.52	2.22	0.065	20.46	9.12	7.341	291.02	0.780

https://doi.org/10.1071/MF18031

River watershed	SITE ID	Year	Richness	Shannon *H′*	Chlorophyll-*a*	Stream order	C-link	River depth	River width	River discharge	Water current velocity	DO	pH	Water temperature	Canopy openness
Yasu River	9	2012	12	1.155	29.123	5	29	6.56	3.82	0.052	23.31	8.89	7.299	291.14	0.780
Yasu River	10	2012	20	2.567	1.179	2	402	8.68	2.86	0.016	9.58	9.53	8.303	289.18	0.081
Yasu River	11	2012	11	2.174	40.085	2	400	8.72	1.08	0.002	3.29	8.74	7.334	291.00	0.780
Yasu River	12	2012	22	2.683	21.025	2	400	30.00	2.72	0.015	2.31	8.26	7.191	290.99	0.780
Yasu River	15	2012	27	2.554	2.398	4	42	17.84	2.20	0.119	35.21	9.28	7.789	291.10	0.633
Yasu River	21	2012	24	1.877	79.074	3	375	42.08	8.88	0.494	17.41	8.32	6.886	290.92	0.780
Yasu River	22	2012	44	2.845	1.031	3	98	29.88	3.42	0.196	20.71	9.2	7.896	290.87	0.633
Yasu River	23	2012	19	1.701	88.433	1	379	12.40	2.50	0.021	11.72	8.79	7.039	290.81	0.780
Yasu River	24	2012	33	2.661	18.931	3	110	19.12	4.00	0.240	34.16	8.93	6.969	290.96	0.603
Yasu River	28	2012	21	2.893	4.760	2	400	26.20	2.86	0.057	9.18	10.25	7.949	290.67	0.603
Yasu River	29	2012	24	2.881	3.179	1	403	14.68	3.62	0.170	33.99	9.97	7.92	289.43	0.754
Yasu River	31	2012	10	2.178	0.710	2	394	5.88	1.10	0.017	27.75	9.92	7.291	290.47	0.603
Yasu River	34	2012	17	2.570	9.105	3	305	30.00	3.28	0.235	26.80	10.02	7.333	290.74	0.693
Yasu River	35	2012	12	1.851	33.891	3	313	19.28	2.54	0.107	24.51	9.96	7.214	290.70	0.693
Yasu River	38	2012			5.030			36.56	9.50	0.670	23.55	10.81	7.587	290.61	0.633
Yasu River	39	2012	11	0.800	104.998	1	403	16.32	2.22	0.021	7.73	8.33	7.225	290.60	0.603
Yasu River	44	2012	8	1.787	32.851	2	397	26.64	4.26	0.035	3.28	9.37	7.273	290.49	0.603
Yasu River	45	2012	28	2.705	8.630	4	376	22.96	11.22	0.339	16.19	10.4	7.351	290.48	0.633
Yasu River	52	2012	20	2.837	26.672	2	402			4.458					
Yasu River	54	2012	21	2.592	9.501	4	329	26.08	30.80	2.528	32.20	8.78	7.361	290.22	0.693
Yasu River	55	2012	16	2.578	6.229	4	354	19.08	14.20	0.991	41.27	9.59	7.556	290.17	0.693
Yasu River	56	2012	10	2.254	5.046	1	403	3.96	1.18	0.004	9.91	9.47	7.389	288.61	0.081
Yasu River	57	2012	23	2.390	0.853	1	403	3.64	1.18	0.006	16.92	9.57	7.597	289.06	0.081
Yasu River	61	2012	16	2.651	0.653	4	374	17.24	7.42	0.680	54.96	9.51	7.443	288.98	0.754
Yasu River	62	2012	26	2.596	1.521	2	349	12.16	2.98	0.051	18.42	9.28		288.75	0.754
Yasu River	64	2012	24	2.058	11.160	4	356	43.52	14.50	2.264	37.80	9.19		288.60	0.754
Yasu River	70	2012	18	1.955	0.290	2	397	12.72	4.14	0.064	16.33	9.69		286.01	0.081
Yasu River	201	2012	9	2.091	0.325	3	394	9.08	6.10	0.234	46.39	9.56		286.91	0.081

Table S2. Correlation matrix of physicochemical environmental variables

The variables used in the final analyses are marked in bold

Variable	Stream order	**C-link**	**River depth**	River width	River discharge	**Water current velocity**	**Dissolved oxygen**	**pH**	**Water temperature**	Canopy openness
Stream order	1									
C-link	–0.387	1								
River depth	0.651	–0.188	1							
River width	0.724	–0.141	0.699	1						
River discharge	0.652	–0.093	0.714	0.920	1					
Water current velocity	0.512	–0.324	0.456	0.568	0.654	1				
Dissolved oxygen	–0.098	0.042	–0.005	–0.070	–0.042	0.283	1			
pH	–0.028	0.096	–0.074	–0.035	–0.004	–0.053	0.172	1		
Water temperature	0.165	0.218	0.096	0.006	–0.044	–0.430	–0.270	0.108	1	
Canopy openness	0.389	0.100	0.247	0.232	0.230	–0.065	–0.322	–0.042	0.719	1
Chlorophyll-*a*	–0.178	0.259	–0.021	–0.144	–0.161	–0.400	–0.514	–0.313	0.484	0.364

https://doi.org/10.1071/MF18031

Table S3. Species list of benthic macroinvertebrates in the Ado River and Yasu River watersheds

Order	Family	Species	Ado River	Yasu River
Tricladida	Dugesiidae	*Dugesia japonica*	V	V
		Girardia tigrina		V
		Dugesiidae gen. sp.		
Gordioida	Chordodidae	*Chordodes* sp.	V	V
Architaenioglossa	Viviparidae	*Sinotaia quadrata histrica*		V
Discopoda	Pleuroceridae	*Semisulcospira libertina*	V	V
		Semisulcospira reiniana		V
	Hydrobiidae	*Potamopyrgus antipodarum*		
	Assimineidae	*Paludinassiminea debilis*		V
Basommatophora	Ancylidae	*Laevapex nipponica*	V	V
	Lymnaeidae	*Fossaria ollula*		V
	Physidae	*Physa acuta*	V	V
Veneroida	Corbiculidae	*Corbicula* sp.	V	V
Haplotaxida	Haplotaxidae	Haplotaxidae gen. sp.		
Tubificida	Naididae	*Branchiodrilus* sp.		V
		Branchiura sowerbyi		V
		Nais sp.		V
		Paranais sp.	V	
		Pristina sp.		V
		Naididae gen. sp.	V	V
		Naididae gen. spp.	V	V
Lumbricida	Lumbricidae	Lumbricidae gen. sp.		
	Megascolecidae	Megascolecidae gen. sp.		V
Rhynchobdellida	Glossiphoniidae	Glossiphoniidae gen. sp.		
Arhynchobdellida	Erpobdellidae	*Dina lineata*	V	
		Erpobdella octoculata	V	V
		Erpobdella testacea		V
		Erpobdellidae gen. sp.		V
	Salifidae	*Odontobdella blanchardi*		
Amphipoda	Crangonyctidae	*Crangonyx floridanus*		V
	Gammaridae	*Gammarus nipponensis*		V
Isopoda	Asellidae	*Asellus hilgendorfi hilgendorfi*	V	V
Decapoda	Atyidae	*Neocaridina denticulata*		V
	Cambaridae	*Procambarus clarkii*		V
	Potamidae	*Geothelphusa dehaani*	V	V
Ephemeroptera	Ameletidae	*Ameletus* sp.	V	
	Baetidae	*Acentrella gnom*	V	V
		Alainites yoshinensis	V	V
		Baetiella japonica	V	V
		Baetis sahoensis	V	V
		Baetis taiwanensis	V	V
		Baetis thermicus	V	V
		Baetis sp. *J*		V
		Baetis sp.	V	V
		Baetis spp.	V	
		Labiobaetis atrebatinus orientalis	V	V
		Nigrobaetis chocoratus	V	V
		Tenuibaetis parvipterus		
		Tenuibaetis flexifemora	V	V
		Baetidae gen. sp.		V
		Baetidae gen. spp.		
	Heptageniidae	*Ecdyonurus bajkovae*	V	
		Ecdyonurus kibunensis	V	V
		Ecdyonurus tigris		V
		Ecdyonurus tobiironis	V	V
		Ecdyonurus yoshidae		V
		Ecdyonurus sp.	V	

Order	Family	Species	Ado River	Yasu River
		Epeorus curvatulus		V
		Epeorus sp.	V	V
		Epeorus nipponicus		V
		Epeorus sp.	V	
		Heptagenia sp.		V
		Rhithrogena tetrapunctigera	V	
		Rhithrogena sp.	V	V
	Isonychiidae	*Isonychia japonica*	V	V
	Leptophlebiidae	*Choroterpes altioculus*	V	V
		Paraleptophlebia japonica	V	
		Paraleptophlebia sp.	V	V
	Ephemeridae	*Ephemera japonica*	V	V
		Ephemera orientalis		V
		Ephemera strigata	V	V
	Polymitarcyidae	*Ephoron shigae*		
	Potamanthidae	*Potamanthus formosus*	V	V
	Ephemerellidae	*Cincticostella nigra*		V
		Cincticostella sp.	V	
		Drunella cryptomeria		
		Drunella ishiyamana		
		Drunella sachalinensis		
		Drunella sp.	V	
		Ephacerella longicaudata	V	
		Ephemerella cornuta		
		Ephemerella imanishii		
		Ephemerella ishiwatai	V	
		Ephemerella setigera	V	V
		Torleya japonica	V	V
		Uracanthella punctisetae	V	V
		Ephemerellidae gen. spp.		
	Caenidae	*Caenis* sp.		V
Odonata	Calopterygidae	*Calopteryx atrata*		V
	Epiophlebiidae	*Epiophlebia superstes*	V	
	Gomphidae	*Davidius* sp.		V
		Nihonogomphus viridis		V
		Onychogomphus viridicostus		V
		Sieboldius albardae	V	V
		Sinogomphus flavolimbatus	V	
		Stylogomphus suzukii		V
		Gomphidae gen. sp.		
	Cordulegasteridae	*Anotogaster sieboldii*	V	V
	Corduliidae	*Macromia amphigena amphigena*		
	Libellulidae	*Orthetrum albistylum speciosum*		V
Plecoptera	Capniidae	Capniidae gen. sp.	V	
	Leuctridae	Leuctridae gen. sp.	V	
	Nemouridae	*Amphinemura* sp.	V	V
		Nemoura sp.		V
		Protonemura sp.	V	V
	Peltoperlidae	*Microperla brevicauda*	V	
		Peltoperlidae gen. sp.	V	
	Chloroperlidae	Chloroperlidae gen. sp.	V	V
	Perlidae	*Caroperla pacifica*	V	V
		Gibosia sp.	V	
		Kamimuria sp.	V	V
		Kiotina sp.	V	
		Neoperla sp.	V	V
		Niponiella limbatella	V	
		Oyamia lugubris	V	
		Oyamia sp.		V

Order	Family	Species	Ado River	Yasu River
		Paragnetina sp.		V
		Togoperla sp.	V	V
		Perlinae gen. sp.		V
		Perlinae gen. spp.	V	
		Perlidae gen. sp.		
	Perlodidae	*Isoperla* sp.	V	
		Perlodidae gen. sp.	V	
Hemiptera	Gerridae	*Metrocoris histrio*		V
	Corixidae	*Micronecta* sp.		
	Aphelochiridae	*Aphelocheirus vittatus*	V	V
Megaloptera	Corydalidae	*Parachauliodes continentalis*	V	
		Protohermes grandis	V	V
	Sialidae	*Sialis* sp.		
Neuroptera	Nevrorthidae	Nevrorthidae gen. sp.		V
Trichoptera	Hydropsychidae	*Cheumatopsyche brevilineata*	V	V
		Cheumatopsyche galloisi	V	
		Cheumatopsyche infascia	V	V
		Cheumatopsyche sp.		V
		Diplectrona sp.	V	V
		Hydropsyche albicephala	V	V
		Hydropsyche ancorapunctata	V	V
		Hydropsyche dilatata	V	
		Hydropsyche orientalis	V	V
		Hydropsyche setensis	V	V
		Hydropsyche sp.	V	
		Macrostemum radiatum		V
	Philopotamidae	*Dolophilodes* sp.	V	V
	Polycentropodidae	*Plectrocnemia* sp.	V	
		Polycentropodidae gen. sp.	V	
	Psychomyiidae	*Psychomyia* sp.	V	V
	Stenopsychidae	*Stenopsyche marmorata*	V	V
		Stenopsyche sauteri	V	V
		Stenopsyche sp.	V	V
	Xiphocentridae	*Melanotrichia* sp.	V	
	Glossosomatidae	*Agapetus* sp.	V	V
		Glossosoma sp.	V	V
		Glossosoma spp.		
		Glossosomatidae gen. spp.		V
	Hydrobiosidae	*Apsilochorema sutshanum*	V	V
	Hydroptilidae	*Hydroptila* sp.		V
	Rhyacophilidae	*Rhyacophila brevicephala*		
		Rhyacophila clemens	V	
		Rhyacophila kawamurae	V	V
		Rhyacophila lezeyi	V	V
		Rhyacophila nigrocephala		V
		Rhyacophila shikotsuensis	V	V
		Rhyacophila transquilla	V	V
		Rhyacophila sp.	V	V
		Rhyacophila spp.	V	
	Apataniidae	*Apatania* sp.	V	
	Brachycentridae	*Brachycentrus* sp.	V	
		Micrasema hanasense	V	
		Micrasema sp.		
	Goeridae	*Goera japonica*	V	V
		Goera sp.	V	V
		Larcasia akagiae	V	
	Lepidostomatidae	*Lepidostoma* sp.	V	V
	Leptoceridae	*Ceraclea* sp.	V	
		Mystacides sp.	V	

https://doi.org/10.1071/MF18031

Order	Family	Species	Ado River	Yasu River
		Trichosetodes japonicus		V
		Leptoceridae gen. sp.	V	
	Limnephilidae	*Nothopsyche* sp.		
	Molannidae	*Molanna moesta*		V
	Phryganeidae	*Eubasilissa regina*	V	
	Sericostomatidae	*Gumaga orientalis*	V	V
	Uenoidae	*Uenoa tokunagai*	V	
Lepidoptera	Crambidae	*Potamomusa midas*		V
		Acentropinae gen. sp.	V	
Diptera	Tipulidae	*Antocha* sp.	V	V
		Dicranota sp.	V	V
		Hexatoma sp.	V	V
		Limnophila sp.		
		Ormosia sp.		
		Tipula sp.	V	V
	Psychodidae	Psychodidae gen. sp.		V
	Ceratopogonidae	Ceratopogonidae gen. sp.	V	V
		Ceratopogonidae gen. spp.		
	Chironomidae	*Brillia* sp.	V	V
		Cardiocladius sp.		V
		Chironomus sp.		V
		Cladotanytarsus sp.		V
		Conchapelopia sp.	V	V
		Cryptochironomus sp.	V	V
		Demicryptochironomus sp.		
		Cryptotendipes sp.		V
		Diamesa sp.	V	
		Dicrotendipes sp.		V
		Eukiefferiella sp.		V
		Eurycnemus nozakii		
		Macropelopia sp.		V
		Metriocnemus sp.		
		Microtendipes sp.		V
		Nanocladius sp.	V	
		Orthocladius sp.	V	V
		Orthocladius spp.	V	V
		Pagastia sp.		V
		Chironomidae gen. sp.	V	V
		Chironomidae gen. spp.	V	V
		Parametriocnemus sp.	V	V
		Polypedilum sp.	V	V
		Potthastia longimana	V	V
		Potthastia sp.	V	
		Pseudorthocladius sp.		V
		Rheocricotopus sp.	V	
		Rheopelopia joganflava	V	V
		Rheotanytarsus sp.	V	V
		Stictochironomus sp.		V
		Tanytarsus sp.	V	V
		Tanytarsus spp.		
		Thienemanniella sp.	V	V
		Tvetenia sp.	V	V
	Dixidae	*Dixa* sp.		
9	Simuliidae	*Simulium* sp.	V	V
	Athericidae	*Asuragina caerulescens*		
		Atherix ibis	V	
		Atrichops morimotoi	V	V
		Athericidae gen. sp.	V	
	Stratiomyidae	Stratiomyidae gen. sp.		V

Order	Family	Species	Ado River	Yasu River
	Tabanidae	Tabanidae gen. sp.	V	
	Dolichopodidae	Dolichopodidae gen. sp.	V	V
Coleoptera	Dytiscidae	*Platambus pictipennis*		
	Hydrophilidae	*Laccobius oscillans*		V
		Hydrophilidae gen. sp.	V	
	Scirtidae	*Elodes* sp.		
		Hydrocyphon sp.	V	
	Elmidae	*Dryopomorphus* sp.		
		Grouvellinus nitidus		
		Optioservus nitidus	V	
		Ordobrevia gotoi	V	
		Ordobrevia maculata	V	V
		Stenelmis miyamotoi		
		Stenelmis nipponica		
		Zaitzevia awana		
		Zaitzevia nitida	V	V
		Zaitzevia rivalis	V	
		Zaitzeviaria brevis	V	V
		Zaitzeviaria gotoi		V
		Elminae sp.	V	V
		Elminae spp.		V
	Psephenidae	*Ectopria opaca opaca*	V	V
		Eubrianax granicollis	V	V
		Mataeopsephus japonicus		V
	Lampyridae	*Luciola cruciata*		V
	Erirhinidae	*Lissorhoptrus oryzophilus*		
Acari	–	*Acarina* spp.	V	V

Marine and Freshwater Research, 2019, **70**, 504–512
https://doi.org/10.1071/MF18031

Supplementary material

Assessing alpha and beta diversities of benthic macroinvertebrates and their environmental drivers between watersheds with different levels of habitat transformation in Japan

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Marine and Freshwater Research
https://doi.org/10.1071/MF18031

Table S1. Values of diversities and environment variables used in this study

River watershed	SITE ID	Year	Richness	Shannon *H′*	Chlorophyll-*a*	Stream order	C-link	River depth	River width	River discharge	Water current velocity	DO	pH	Water temperature	Canopy openness
Ado River	1	2014	29	2.201	5.642	2	303	22.56	2.78	0.103	19.57	9.34	6.818	286.18	0.049
Ado River	3	2014	25	2.260	4.185	2	290	8.20	2.92	0.076	34.32	9.18	7.057	286.10	0.125
Ado River	8	2014	18	2.168	0.759	3	283	19.52	7.00	0.543	41.60	9.37	7.29	287.01	0.194
Ado River	9	2014	14	1.839	2.471	4	28	24.76	9.06	1.423	64.60	9.07	7.149	287.35	0.351
Ado River	13	2014	21	2.491	2.342	3	269	35.00	11.70	0.755	29.23	9.33	7.417	287.31	0.034
Ado River	15	2014	22	2.901	0.613	1	300	10.47	2.03	0.054	27.90	9.41	7.328	286.93	0.038
Ado River	17	2014	16	2.207	0.625	3	42	19.48	9.10	0.955	54.14	9.32	7.041	287.31	0.430
Ado River	20	2014	17	2.677	1.529	1	43	7.80	2.02	0.037	25.01	9.31	7.416	287.12	0.175
Ado River	22	2014	16	2.120	0.282	2	22	10.04	6.32	0.365	57.79	9.11	7.422	287.01	0.688
Ado River	23	2014	35	2.890	0.563	3	128	10.84	5.56	0.189	33.54	9.07	7.38	287.18	0.412
Ado River	26	2014	26	2.699	1.923	4	13	25.36	15.24	2.175	59.21	9.34	7.311	287.36	0.069
Ado River	28	2014	15	2.404	0.409	2	303	15.72	5.30	0.104	12.99	9.39	7.495	287.43	0.008
Ado River	30	2014	43	3.275	0.398	5	5	55.00	19.73	4.757	44.78	9.71	7.42	287.62	0.589
Ado River	32	2014	33	2.973	2.601	2	281	12.84	4.46	0.159	33.91	9.97	7.187	287.60	0.032
Ado River	33	2014	43	3.144	0.555	5	293	36.20	33.18	8.051	70.05	9.6	7.227	288.11	0.427
Ado River	35	2014	28	2.686	2.511	2	287	17.56	5.32	0.437	50.24	10.2	7.374	287.38	0.034
Ado River	37	2014	6	1.565	13.147	1	291	18.84	2.58	0.117	26.93	9.54	7.366	287.16	0.013
Ado River	41	2014	21	2.272	0.805	3	144	38.40	14.28	3.340	63.94	10.38	7.421	287.76	0.166
Ado River	42	2014	26	2.639	0.895	2	144	18.36	5.14	0.688	74.79	9.8	7.306	287.53	0.022
Ado River	43	2014	24	2.273	3.452	3	302	36.76	16.12	5.066	86.25	10.31	7.259	287.92	0.609
Ado River	45	2014	26	1.941	3.117	2	272	24.12	5.98	0.719	52.04	10.07	7.161	287.50	0.044
Ado River	47	2014	19	2.445	2.289	2	270	18.20	4.44	0.488	63.64	10.24	7.586	287.50	0.306
Ado River	48	2014	22	2.690	1.082	3	283	32.68	13.14	3.850	94.66	10.22	7.419	287.89	0.104
Ado River	51	2014	18	2.691	1.023	4	282	39.32	24.00	7.658	81.91	10.32	6.929	288.28	0.707
Ado River	52	2014	37	2.875	3.493	1	169	25.92	3.50	0.087	10.01	10.38	7.213	289.31	0.231
Ado River	53	2014	28	3.009	6.008	5	250	39.64	62.38	15.342	68.65	9.22	7.395	289.33	0.686
Ado River	57	2014	24	2.652	1.405	5	267	46.95	32.72	13.228	100.22	9.01	7.486	288.93	0.660
Ado River	59	2014	44	3.018	7.350	2	277	8.44	3.20	0.047	18.08	9.6	6.956	289.55	0.635
Ado River	60	2014	28	2.561	3.309	2	305	10.28	2.22	0.050	30.62	9.79	7.156	288.40	0.591
Ado River	63	2014	14	2.553	3.195	5	305	62.16	34.50	12.832	67.89	8.71	7.523	288.99	0.787
Yasu River	3	2012	17	2.467	22.883	5	1	53.64	29.20	4.881	32.31	9.25	7.129	292.49	0.633
Yasu River	8	2012	30	2.741	2.486	2	402	15.52	2.22	0.065	20.46	9.12	7.341	291.02	0.780

River watershed	SITE ID	Year	Richness	Shannon *H′*	Chlorophyll-*a*	Stream order	C-link	River depth	River width	River discharge	Water current velocity	DO	pH	Water temperature	Canopy openness
Yasu River	9	2012	12	1.155	29.123	5	29	6.56	3.82	0.052	23.31	8.89	7.299	291.14	0.780
Yasu River	10	2012	20	2.567	1.179	2	402	8.68	2.86	0.016	9.58	9.53	8.303	289.18	0.081
Yasu River	11	2012	11	2.174	40.085	2	400	8.72	1.08	0.002	3.29	8.74	7.334	291.00	0.780
Yasu River	12	2012	22	2.683	21.025	2	400	30.00	2.72	0.015	2.31	8.26	7.191	290.99	0.780
Yasu River	15	2012	27	2.554	2.398	4	42	17.84	2.20	0.119	35.21	9.28	7.789	291.10	0.633
Yasu River	21	2012	24	1.877	79.074	3	375	42.08	8.88	0.494	17.41	8.32	6.886	290.92	0.780
Yasu River	22	2012	44	2.845	1.031	3	98	29.88	3.42	0.196	20.71	9.2	7.896	290.87	0.633
Yasu River	23	2012	19	1.701	88.433	1	379	12.40	2.50	0.021	11.72	8.79	7.039	290.81	0.780
Yasu River	24	2012	33	2.661	18.931	3	110	19.12	4.00	0.240	34.16	8.93	6.969	290.96	0.603
Yasu River	28	2012	21	2.893	4.760	2	400	26.20	2.86	0.057	9.18	10.25	7.949	290.67	0.603
Yasu River	29	2012	24	2.881	3.179	1	403	14.68	3.62	0.170	33.99	9.97	7.92	289.43	0.754
Yasu River	31	2012	10	2.178	0.710	2	394	5.88	1.10	0.017	27.75	9.92	7.291	290.47	0.603
Yasu River	34	2012	17	2.570	9.105	3	305	30.00	3.28	0.235	26.80	10.02	7.333	290.74	0.693
Yasu River	35	2012	12	1.851	33.891	3	313	19.28	2.54	0.107	24.51	9.96	7.214	290.70	0.693
Yasu River	38	2012			5.030			36.56	9.50	0.670	23.55	10.81	7.587	290.61	0.633
Yasu River	39	2012	11	0.800	104.998	1	403	16.32	2.22	0.021	7.73	8.33	7.225	290.60	0.603
Yasu River	44	2012	8	1.787	32.851	2	397	26.64	4.26	0.035	3.28	9.37	7.273	290.49	0.603
Yasu River	45	2012	28	2.705	8.630	4	376	22.96	11.22	0.339	16.19	10.4	7.351	290.48	0.633
Yasu River	52	2012	20	2.837	26.672	2	402			4.458					
Yasu River	54	2012	21	2.592	9.501	4	329	26.08	30.80	2.528	32.20	8.78	7.361	290.22	0.693
Yasu River	55	2012	16	2.578	6.229	4	354	19.08	14.20	0.991	41.27	9.59	7.556	290.17	0.693
Yasu River	56	2012	10	2.254	5.046	1	403	3.96	1.18	0.004	9.91	9.47	7.389	288.61	0.081
Yasu River	57	2012	23	2.390	0.853	1	403	3.64	1.18	0.006	16.92	9.57	7.597	289.06	0.081
Yasu River	61	2012	16	2.651	0.653	4	374	17.24	7.42	0.680	54.96	9.51	7.443	288.98	0.754
Yasu River	62	2012	26	2.596	1.521	2	349	12.16	2.98	0.051	18.42	9.28		288.75	0.754
Yasu River	64	2012	24	2.058	11.160	4	356	43.52	14.50	2.264	37.80	9.19		288.60	0.754
Yasu River	70	2012	18	1.955	0.290	2	397	12.72	4.14	0.064	16.33	9.69		286.01	0.081
Yasu River	201	2012	9	2.091	0.325	3	394	9.08	6.10	0.234	46.39	9.56		286.91	0.081

Table S2. Correlation matrix of physicochemical environmental variables

The variables used in the final analyses are marked in bold

Variable	Stream order	**C-link**	**River depth**	River width	River discharge	**Water current velocity**	**Dissolved oxygen**	**pH**	**Water temperature**	Canopy openness
Stream order	1									
C-link	–0.387	1								
River depth	0.651	–0.188	1							
River width	0.724	–0.141	0.699	1						
River discharge	0.652	–0.093	0.714	0.920	1					
Water current velocity	0.512	–0.324	0.456	0.568	0.654	1				
Dissolved oxygen	–0.098	0.042	–0.005	–0.070	–0.042	0.283	1			
pH	–0.028	0.096	–0.074	–0.035	–0.004	–0.053	0.172	1		
Water temperature	0.165	0.218	0.096	0.006	–0.044	–0.430	–0.270	0.108	1	
Canopy openness	0.389	0.100	0.247	0.232	0.230	–0.065	–0.322	–0.042	0.719	1
Chlorophyll-*a*	–0.178	0.259	–0.021	–0.144	–0.161	–0.400	–0.514	–0.313	0.484	0.364

Table S3. Species list of benthic macroinvertebrates in the Ado River and Yasu River watersheds

Order	Family	Species	Ado River	Yasu River
Tricladida	Dugesiidae	*Dugesia japonica*	V	V
		Girardia tigrina		V
		Dugesiidae gen. sp.		
Gordioida	Chordodidae	*Chordodes* sp.	V	V
Architaenioglossa	Viviparidae	*Sinotaia quadrata histrica*		V
Discopoda	Pleuroceridae	*Semisulcospira libertina*	V	V
		Semisulcospira reiniana		V
	Hydrobiidae	*Potamopyrgus antipodarum*		
	Assimineidae	*Paludinassiminea debilis*		V
Basommatophora	Ancylidae	*Laevapex nipponica*	V	V
	Lymnaeidae	*Fossaria ollula*		V
	Physidae	*Physa acuta*	V	V
Veneroida	Corbiculidae	*Corbicula* sp.	V	V
Haplotaxida	Haplotaxidae	Haplotaxidae gen. sp.		
Tubificida	Naididae	*Branchiodrilus* sp.		V
		Branchiura sowerbyi		V
		Nais sp.		V
		Paranais sp.	V	
		Pristina sp.		V
		Naididae gen. sp.	V	V
		Naididae gen. spp.	V	V
Lumbricida	Lumbricidae	Lumbricidae gen. sp.		
	Megascolecidae	Megascolecidae gen. sp.		V
Rhynchobdellida	Glossiphoniidae	Glossiphoniidae gen. sp.		
Arhynchobdellida	Erpobdellidae	*Dina lineata*	V	
		Erpobdella octoculata	V	V
		Erpobdella testacea		V
		Erpobdellidae gen. sp.		V
	Salifidae	*Odontobdella blanchardi*		
Amphipoda	Crangonyctidae	*Crangonyx floridanus*		V
	Gammaridae	*Gammarus nipponensis*		V
Isopoda	Asellidae	*Asellus hilgendorfi hilgendorfi*	V	V
Decapoda	Atyidae	*Neocaridina denticulata*		V
	Cambaridae	*Procambarus clarkii*		V
	Potamidae	*Geothelphusa dehaani*	V	V
Ephemeroptera	Ameletidae	*Ameletus* sp.	V	
	Baetidae	*Acentrella gnom*	V	V
		Alainites yoshinensis	V	V
		Baetiella japonica	V	V
		Baetis sahoensis	V	V
		Baetis taiwanensis	V	V
		Baetis thermicus	V	V
		Baetis sp. *J*		V
		Baetis sp.	V	V
		Baetis spp.	V	
		Labiobaetis atrebatinus orientalis	V	V
		Nigrobaetis chocoratus	V	V
		Tenuibaetis parvipterus		
		Tenuibaetis flexifemora	V	V
		Baetidae gen. sp.		V
		Baetidae gen. spp.		
	Heptageniidae	*Ecdyonurus bajkovae*	V	
		Ecdyonurus kibunensis	V	V
		Ecdyonurus tigris		V
		Ecdyonurus tobiironis	V	V
		Ecdyonurus yoshidae		V
		Ecdyonurus sp.	V	

Order	Family	Species	Ado River	Yasu River
		Epeorus curvatulus		V
		Epeorus sp.	V	V
		Epeorus nipponicus		V
		Epeorus sp.	V	
		Heptagenia sp.		V
		Rhithrogena tetrapunctigera	V	
		Rhithrogena sp.	V	V
	Isonychiidae	*Isonychia japonica*	V	V
	Leptophlebiidae	*Choroterpes altioculus*	V	V
		Paraleptophlebia japonica	V	
		Paraleptophlebia sp.	V	V
	Ephemeridae	*Ephemera japonica*	V	V
		Ephemera orientalis		V
		Ephemera strigata	V	V
	Polymitarcyidae	*Ephoron shigae*		
	Potamanthidae	*Potamanthus formosus*	V	V
	Ephemerellidae	*Cincticostella nigra*		V
		Cincticostella sp.	V	
		Drunella cryptomeria		
		Drunella ishiyamana		
		Drunella sachalinensis		
		Drunella sp.	V	
		Ephacerella longicaudata	V	
		Ephemerella cornuta		
		Ephemerella imanishii		
		Ephemerella ishiwatai	V	
		Ephemerella setigera	V	V
		Torleya japonica	V	V
		Uracanthella punctisetae	V	V
		Ephemerellidae gen. spp.		
	Caenidae	*Caenis* sp.		V
Odonata	Calopterygidae	*Calopteryx atrata*		V
	Epiophlebiidae	*Epiophlebia superstes*	V	
	Gomphidae	*Davidius* sp.		V
		Nihonogomphus viridis		V
		Onychogomphus viridicostus		V
		Sieboldius albardae	V	V
		Sinogomphus flavolimbatus	V	
		Stylogomphus suzukii		V
		Gomphidae gen. sp.		
	Cordulegasteridae	*Anotogaster sieboldii*	V	V
	Corduliidae	*Macromia amphigena amphigena*		
	Libellulidae	*Orthetrum albistylum speciosum*		V
Plecoptera	Capniidae	Capniidae gen. sp.	V	
	Leuctridae	Leuctridae gen. sp.	V	
	Nemouridae	*Amphinemura* sp.	V	V
		Nemoura sp.		V
		Protonemura sp.	V	V
	Peltoperlidae	*Microperla brevicauda*	V	
		Peltoperlidae gen. sp.	V	
	Chloroperlidae	Chloroperlidae gen. sp.	V	V
	Perlidae	*Caroperla pacifica*	V	V
		Gibosia sp.	V	
		Kamimuria sp.	V	V
		Kiotina sp.	V	
		Neoperla sp.	V	V
		Niponiella limbatella	V	
		Oyamia lugubris	V	
		Oyamia sp.		V

Order	Family	Species	Ado River	Yasu River
		Paragnetina sp.		V
		Togoperla sp.	V	V
		Perlinae gen. sp.		V
		Perlinae gen. spp.	V	
		Perlidae gen. sp.		
	Perlodidae	*Isoperla* sp.	V	
		Perlodidae gen. sp.	V	
Hemiptera	Gerridae	*Metrocoris histrio*		V
	Corixidae	*Micronecta* sp.		
	Aphelochiridae	*Aphelocheirus vittatus*	V	V
Megaloptera	Corydalidae	*Parachauliodes continentalis*	V	
		Protohermes grandis	V	V
	Sialidae	*Sialis* sp.		
Neuroptera	Nevrorthidae	Nevrorthidae gen. sp.		V
Trichoptera	Hydropsychidae	*Cheumatopsyche brevilineata*	V	V
		Cheumatopsyche galloisi	V	
		Cheumatopsyche infascia	V	V
		Cheumatopsyche sp.		V
		Diplectrona sp.	V	V
		Hydropsyche albicephala	V	V
		Hydropsyche ancorapunctata	V	V
		Hydropsyche dilatata	V	
		Hydropsyche orientalis	V	V
		Hydropsyche setensis	V	V
		Hydropsyche sp.	V	
		Macrostemum radiatum		V
	Philopotamidae	*Dolophilodes* sp.	V	V
	Polycentropodidae	*Plectrocnemia* sp.	V	
		Polycentropodidae gen. sp.	V	
	Psychomyiidae	*Psychomyia* sp.	V	V
	Stenopsychidae	*Stenopsyche marmorata*	V	V
		Stenopsyche sauteri	V	V
		Stenopsyche sp.	V	V
	Xiphocentridae	*Melanotrichia* sp.	V	
	Glossosomatidae	*Agapetus* sp.	V	V
		Glossosoma sp.	V	V
		Glossosoma spp.		
		Glossosomatidae gen. spp.		V
	Hydrobiosidae	*Apsilochorema sutshanum*	V	V
	Hydroptilidae	*Hydroptila* sp.		V
	Rhyacophilidae	*Rhyacophila brevicephala*		
		Rhyacophila clemens	V	
		Rhyacophila kawamurae	V	V
		Rhyacophila lezeyi	V	V
		Rhyacophila nigrocephala		V
		Rhyacophila shikotsuensis	V	V
		Rhyacophila transquilla	V	V
		Rhyacophila sp.	V	V
		Rhyacophila spp.	V	
	Apataniidae	*Apatania* sp.	V	
	Brachycentridae	*Brachycentrus* sp.	V	
		Micrasema hanasense	V	
		Micrasema sp.		
	Goeridae	*Goera japonica*	V	V
		Goera sp.	V	V
		Larcasia akagiae	V	
	Lepidostomatidae	*Lepidostoma* sp.	V	V
	Leptoceridae	*Ceraclea* sp.	V	
		Mystacides sp.	V	

Order	Family	Species	Ado River	Yasu River
		Trichosetodes japonicus		V
		Leptoceridae gen. sp.	V	
	Limnephilidae	*Nothopsyche* sp.		
	Molannidae	*Molanna moesta*		V
	Phryganeidae	*Eubasilissa regina*	V	
	Sericostomatidae	*Gumaga orientalis*	V	V
	Uenoidae	*Uenoa tokunagai*	V	
Lepidoptera	Crambidae	*Potamomusa midas*		V
		Acentropinae gen. sp.	V	
Diptera	Tipulidae	*Antocha* sp.	V	V
		Dicranota sp.	V	V
		Hexatoma sp.	V	V
		Limnophila sp.		
		Ormosia sp.		
		Tipula sp.	V	V
	Psychodidae	Psychodidae gen. sp.		V
	Ceratopogonidae	Ceratopogonidae gen. sp.	V	V
		Ceratopogonidae gen. spp.		
	Chironomidae	*Brillia* sp.	V	V
		Cardiocladius sp.		V
		Chironomus sp.		V
		Cladotanytarsus sp.		V
		Conchapelopia sp.	V	V
		Cryptochironomus sp.	V	V
		Demicryptochironomus sp.		
		Cryptotendipes sp.		V
		Diamesa sp.	V	
		Dicrotendipes sp.		V
		Eukiefferiella sp.		V
		Eurycnemus nozakii		
		Macropelopia sp.		V
		Metriocnemus sp.		
		Microtendipes sp.		V
		Nanocladius sp.	V	
		Orthocladius sp.	V	V
		Orthocladius spp.	V	V
		Pagastia sp.		V
		Chironomidae gen. sp.	V	V
		Chironomidae gen. spp.	V	V
		Parametriocnemus sp.	V	V
		Polypedilum sp.	V	V
		Potthastia longimana	V	V
		Potthastia sp.	V	
		Pseudorthocladius sp.		V
		Rheocricotopus sp.	V	
		Rheopelopia joganflava	V	V
		Rheotanytarsus sp.	V	V
		Stictochironomus sp.		V
		Tanytarsus sp.	V	V
		Tanytarsus spp.		
		Thienemanniella sp.	V	V
		Tvetenia sp.	V	V
	Dixidae	*Dixa* sp.		
9	Simuliidae	*Simulium* sp.	V	V
	Athericidae	*Asuragina caerulescens*		
		Atherix ibis	V	
		Atrichops morimotoi	V	V
		Athericidae gen. sp.	V	
	Stratiomyidae	Stratiomyidae gen. sp.		V

Order	Family	Species	Ado River	Yasu River
	Tabanidae	Tabanidae gen. sp.	V	
	Dolichopodidae	Dolichopodidae gen. sp.	V	V
Coleoptera	Dytiscidae	*Platambus pictipennis*		
	Hydrophilidae	*Laccobius oscillans*		V
		Hydrophilidae gen. sp.	V	
	Scirtidae	*Elodes* sp.		
		Hydrocyphon sp.	V	
	Elmidae	*Dryopomorphus* sp.		
		Grouvellinus nitidus		
		Optioservus nitidus	V	
		Ordobrevia gotoi	V	
		Ordobrevia maculata	V	V
		Stenelmis miyamotoi		
		Stenelmis nipponica		
		Zaitzevia awana		
		Zaitzevia nitida	V	V
		Zaitzevia rivalis	V	
		Zaitzeviaria brevis	V	V
		Zaitzeviaria gotoi		V
		Elminae sp.	V	V
		Elminae spp.		V
	Psephenidae	*Ectopria opaca opaca*	V	V
		Eubrianax granicollis	V	V
		Mataeopsephus japonicus		V
	Lampyridae	*Luciola cruciata*		V
	Erirhinidae	*Lissorhoptrus oryzophilus*		
Acari	–	*Acarina* spp.	V	V