Title:

Unique habitat and macroinvertebrate assemblage structures in spring-fed streams: a comparison among lowland tributaries and mainstreams in northern Japan

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Abstract

The stable flow and temperature regimes of spring-fed streams are distinct from the dynamic regimes of other streams. We investigated differences in habitat and macroinvertebrate assemblages among a spring-fed tributary, an adjacent non-spring-fed tributary, and mainstream reaches. Current velocity was slowest in the spring-fed reach and percent fine sediment deposition was 3.8–13.9 times higher than in non-spring streams. Mean periphyton biomass was greatest in the mainstream reach where canopy openness was greatest, whereas the standing stock of detritus was greatest in the spring-fed reach. These results suggest that the stable flow regime in the spring-fed stream leads to the accumulation of fine sediment and detritus on the streambed. Oligochaeta and chironomids, which are burrower-gatherers, were remarkably abundant in the spring-fed reach and total macroinvertebrate abundance was 3.4–12.2 times greater than in the other reaches. Sprawler-grazer ephemeropterans were abundant in the mainstream reaches, likely due to higher primary productivity. *Allomyia* sp, which depend on cool spring-fed habitats, were found only in the spring-fed reach. The macroinvertebrate assemblage in the spring-fed reach was characterized by numerous burrowers, collector-gatherers, and crenobiont taxa, highlighting the contribution of these habitats to beta diversity in river networks.

Keywords

Chironomidae, Fine sediment, Flow regime, Groundwater, River ecosystem

Introduction

Groundwater flow into river ecosystems depends on underground flow pathways and aboveground topographic features (Allan and Castillo 2007). Although apportioning stream water to runoff and groundwater sources is generally difficult, streams that are sustained by a significant amount of permanent groundwater discharge are typically referred to as spring-fed streams. Because groundwater flow is rarely affected by rapid fluctuations resulting from rainfall, snowmelt, or seasonal and diurnal changes in air temperature, spring-fed streams are characterized by stable water flow and temperature regimes (Mattson et al. 1995; Sear et al. 1999; Lusardi et al. 2016). Thus, spring-fed streams are distinct from the dynamic flow and temperature regimes that characterize non-spring-fed streams.

Flow regime is one of the most influential factors determining the structure of streambed substrates (Davis and Barmuta 1989). The frequency and magnitude of high flows are directly related to sediment runoff; stream substrate components are sequentially flushed from smaller to larger particles under high flows (Richards 2004). Streams with frequent high flows typically have a coarse streambed structure relative to those with less variable flow regimes. The substrates of spring-fed streams are dominated by fine sediments, owing to their stable flow regimes (Sear et al. 1999). Spring-fed streams in lowland regions may have particularly fine streambeds due to low channel gradients. Streambed particle size is a major determinant of the benthic macroinvertebrate community and its structure (Richards and Bacon 1994; Wood and Armitage 1997). For example, the relative density of burrowers and gatherers may increase with fine sediment deposition (Rabení et al. 2005). These groups are well

suited to depositional habitats because these habitats provide organic matter as well as burrowing spaces. Spring-fed streams may therefore house unique community assemblages, dominated by burrowers and gatherers, relative to adjacent non-spring-fed streams that have less fine sediment content.

Water temperature in spring-fed streams is highly stable; it is typically cold in summer and warm in winter (Mattson et al. 1995). Previous studies have reported that these stable water temperature regimes provide refugia for aquatic fauna to avoid severe hot (Nakagawa et al. 2015) and cold seasonal conditions (Inoue and Ishigaki 1968). Moreover, Sun *et al*. (in press) suggested that most indicator species of spring-fed habitats may be stenothermal. This further suggests that the narrow range of water temperatures in spring-fed streams may lead to the formation of unique assemblages of crenobiont and/or crenophilous species (Lusardi et al. 2016).

The stable flow and temperature regimes of spring-fed streams provide unique habitat and macroinvertebrate assemblage structures and subsequently contribute to beta diversity within river networks. However, although more than 16,000 spring-fed sites have been recognized in Japan (Ministry of Environment 2020), the ecosystem functions of these streams remain poorly understood. We assessed differences in habitat characteristics and macroinvertebrate assemblages between spring-fed and adjacent non-spring-fed streams in a lowland area in Japan. Our aim was to elucidate plausible processes explaining these differences and to contribute to the conservation and management of lotic systems under environmental change (e.g., van Vliet et al. 2013; Yin et al. 2018).

Methods

Study area

We assessed streams in the Shubuto River system, located in Kuromatsunai, Hokkaido, Japan (42.64°N, 140.34°E). The Shubuto River basin comprises 367 km^2 of montane and lowland areas; our study focused on the lowland region. The geology is dominated by sandstone and mudstone with Cainozoic fossil seashells and tuff. Mean annual precipitation and air temperature, obtained from the Kuromatsunai automated meteorological data acquisition system station (located 4 km northwest of the site) were 1615.8 mm and 7.5°C, respectively, between 2009 and 2018. Dominant tree species in the riparian zones of the study area were *Salix* spp. and *Quercus crispula*, and dominant understory plants were *Sasa kurilensis* and *Reynoutria sachalinensis*.

The lowland region of the Shubuto River basin includes areas of groundwater discharge such as flowing wells, seepages, and springs. The spring-fed tributary assessed in this study is fed by a significant amount of groundwater discharge (approximately 0.1 m^3/s) that is directly connected to the Shubuto River (Sakai et al. 2020). This tributary has stable flow regime and water temperature compared to adjacent streams (Sakai, unpublished data). We further selected a non-spring-fed tributary and three mainstreams that also drained into the lowland area (Fig. 1). The spring-fed and non-spring-fed tributaries (ST1 and ST2, respectively) were near to each other and had similar flow directions. Three mainstream areas were identified upward of the confluence with St. 2 (ST3), between the confluences with ST1 and ST2 (ST4), and below the confluence with ST1 (ST5, Fig. 1). We used 40-m sections (reaches) in each study site to assess macroinvertebrates and habitat characteristics.

Macroinvertebrate assemblage

We used Surber nets (25 × 25 cm, 0.5-mm mesh) to sample macroinvertebrates from five randomly placed quadrats in each reach in June 2016. Samples containing macroinvertebrates, detritus, and inorganic sediments were placed a tray and the macroinvertebrate and detritus portions were rinsed into unwoven bags, respectively, and immediately preserved in 70% ethanol. Macroinvertebrates were sorted and identified to the lowest possible taxonomic level using a stereomicroscope (SZ61, Olympus, Tokyo, Japan) and keys published by Kawai and Tanida (2005) and Merritt et al. (2008). Following identification, we classified macroinvertebrates by life-form type (burrowers, sprawlers, clingers, and swimmers) and functional feeding group (collector-gatherers, predators, and grazers) based on information from Takemon (2005) and Merritt et al. (2008) as well as our own unpublished data.

Habitat characteristics

Stream width was measured with a measuring tape under base flow conditions at five points in each study reach. We measured water depth and current velocity (repeated 5-s measurements) using a folding scale and an electromagnetic current meter (VE10, Kenek, Tokyo, Japan), respectively, at 15 points in each study reach during macroinvertebrate sampling. Hemispherical photographs were taken in June 2016 at 10 m intervals along the 40 m reaches (five points) to estimate canopy openness. Photographs were taken with a digital camera (D40, Nikon, Tokyo, Japan) equipped with a fisheye lens (4.5 mm F2.8 EX DC Circular Fisheye HSM, Sigma, Kanagawa, Japan) that was fixed horizontally at 1 m above the streambed. Canopy openness (%)

was estimated using CanopOn2 software (Takenaka 2009) from these photographs.

We applied the methods of Sakai et al. (2013) to estimate fine sediment deposition in each study reach by visually estimating the percent cover of fine sediment (<2 mm) on the streambed in each Surber net quadrat (5% increments, five quadrats per reach). We also estimated the periphyton biomass on upper exposed surfaces of five submerged large cobbles in each study reach. Periphyton was collected by brushing material from a 4 cm^2 patch on the upper cobble surface and filtering the resulting slurry through pre-combusted glass-microfiber filters (Whatman GF/F, Maidstone, United Kingdom). Periphyton dry mass and ash-free dry mass (AFDM) were determined by drying the periphyton samples in an oven (FC-610, Advantec, Tokyo, Japan) at 70°C for 24 h and then weighing them, followed by combustion in a muffle furnace (FO300, Yamato Scientific Co., Ltd., Tokyo, Japan) at 500°C for 2 h and re-weighing. Detritus samples were dried at ambient temperature for 3 days, followed by oven drying at 70°C for 3 days and combustion in a muffle furnace at 500°C for 4 h. The AFDM of periphyton and detritus samples were used as measures of periphyton biomass and standing stock of detritus (mg/cm^2), respectively.

Statistical analyses

To determine differences in habitat characteristics and macroinvertebrate assemblages among reaches, we performed multiple comparisons for each environmental variable, the total abundance and taxa richness of macroinvertebrates, and the abundance of dominant taxa (defined as any taxon comprising >1% of the total number of individuals). Prior to comparisons, we tested each variable for homogeneity of variance using Bartlett's test. We used raw values where variables satisfied the homogeneity of

variance assumption and applied a log transformation (log[X+1]) where they did not. We used Tukey-Kramer tests for variables that satisfied the homogeneity of variance assumption. Where both raw and log-transformed values did not satisfy the assumption, we applied a Steel-Dwass test using the Monte Carlo method with 10,000 iterations. Multiple comparisons could not be performed for some variables with excessive zero values. All statistical analyses were performed in R version 3.6.3 (R Core Team 2020). We used the package 'NSM3' (Schneider et al. 2020) for the Steel-Dwass tests.

Results

Stream width was significantly greater in mainstream reaches than in both tributaries (Table 1) and ST1 was wider than ST2. Correspondingly, canopy openness was highest in the mainstream reaches. Differences in water depth were similar to those for stream width. Current velocity was slowest in ST1 (13 cm/s) and ranged between 24 and 59 cm/s in the remaining reaches. The percent cover of fine sediment was highest in ST1; mean cover was approximately 10 times greater than those of ST3–5 and approximately four times higher than that of ST2 (Table 1). Although we did not observe clear statistical differences in periphyton biomass or standing stock of detritus among reaches, mean periphyton biomass was greatest in ST4, which also had the most open canopy, and mean standing stock of detritus was highest in ST1, which had the slowest current velocity (Table 1).

The total abundance and taxa richness of macroinvertebrates were both highest in ST1 and lowest in ST2 (Table 2) and were relatively similar among the mainstream

reaches. For life-form types, the four most abundant burrower taxa (Oligochaeta, *Pagastia* spp., Chironomidae gen. spp. and Chironominae spp.) and Tanypodinae sp. was most abundant in ST1, but some dominant taxa (*Dicranota* spp., Orthocladiinae sp. and *Paraleptophlebia japonica*) were more abundant in other reaches. Sprawlers were typically the most abundant group in the mainstream reaches. For functional feeding groups, the four most dominant collector-gatherer taxa (Oligochaeta, *Pagastia* spp., Chironomidae gen. spp. and Chironominae spp.) were most abundant in ST1 (Table 2). Excluding two trichopteran grazers that were dominant in ST1 (*Apatania* sp. and *Allomyia* sp.), all grazer taxa reached their maximum abundance in the mainstream reaches. We note that *Allomyia* sp. was only found in ST1 (Table 2).

Discussion

Spring-fed streams in lowlands rarely have steep, deep channels (Sear et al. 1999) and their flow regimes are stable (Mattson et al. 1995; Lusardi et al. 2016). Sakai et al. (2020) indicated that the spring-fed tributary assessed here maintained a stable flow regime even during rainfall events, implying that sediment runoff during floods likely occurs less in ST1 than in other non-spring-fed reaches. Therefore, the significantly greater cover of fine sediment and higher standing stock of detritus observed in ST1 are likely the result of its stable flow regime.

Oligochaeta and most of the chironomid taxa were remarkably abundant in ST1. As a result, the total macroinvertebrate abundance was 3.4–12.2 times greater in this tributary than in the other reaches. The relative abundance of burrowers increases with

fine sediment deposition (e.g., Zweig and Rabení 2001; Rabení et al. 2005), and our findings suggest that the fine substrate found in spring-fed streams provides attractive habitat for burrower species. Moreover, the stable flow regimes of spring-fed streams may relate to less disturbance to macroinvertebrates during flood events (e.g., Fritz and Dodds 2004; Robinson et al. 2004). Because the burrower species that dominated in ST1 typically gather and feed on fine detritus (i.e., collector-gatherers), spring-fed environments clearly provide adequate food resources for this group. The dominance of burrowing collector-gatherers at ST1 implies that the food web is detrital-based and therefore differs from those of nearby non-spring-fed streams.

In contrast to our results, previous studies have reported that spring-fed streams often have lush macrophytes (e.g., Sherwood et al. 2000; Katagiri et al. 2011) and are accordingly dominated by herbivorous invertebrates (Takemon 2010). The streambed of the spring-fed stream we studied lacked macrophytes and had a low abundance of grazers relative to collector-gatherers, perhaps because of limited light conditions. Food web structures in spring-fed streams are probably site-specific and tightly associated with environmental factors that affect the growth of macrophytes.

Wider streams and more open canopies in the mainstream reaches imply higher productivity, but differences in periphyton biomass were not significantly different between mainstreams and tributaries. However, the dominance of ephemeropteran grazers in mainstream reaches supports their higher productivity, because higher periphyton productivity is associated with greater occupancy by grazers (e.g., Feminella et al. 1989; Hill et al. 1995). Therefore, grazing-based food webs may be more pronounced in mainstreams and the dominance of grazer taxa and associated consumption of periphyton may have led to the lack of differences in periphyton

biomass among reaches. The dominance of sprawlers in mainstreams is attributable to their adaptability to fast-current habitats.

Given that the basal food resources and macroinvertebrate communities differed between the spring-fed tributary and the adjacent mainstream reaches, we suggest that the confluence of these streams may tangle the structure of the food web such that the abundant macroinvertebrates in the spring-fed tributary may subsidize predatory animals, such as fishes, inhabiting the surrounding streams (cf. Uno and Power 2015). Moreover, the stable flow regime and water temperature in spring-fed streams provide refugia during flood events (Sakai et al. 2020) and thermal stresses (Inoue and Ishigaki 1968). Terui et al. (2018) reported that complex branches in river networks can increase metapopulation stability for fish. Spring-fed streams may also contribute to such stabilities in stream animal communities. Given that spring-fed habitats are ubiquitous and distributed across Japan, examining the ecosystem functions of these streams in nutrient cycling and as refugia is an important topic in river ecosystem conservation. Given that the climate crisis relates to extreme runoff events (Yin et al. 2018) and severe thermal stress (van Vliet et al. 2013), these streams may be vital to conservation efforts.

Two sprawler-grazer trichopteran species (*Allomyia* sp. and *Apatania* sp.) that can be considered crenobiont and/or crenophilous were dominant in ST1. In Japan, *Allomyia* species occur only in Hokkaido, and are preferentially found in cool spring-fed habitats (Nishimoto and Kuhara 2001; Kawai and Tanida 2005). *Allomyia* sp. was found only in the spring-fed tributary in this study. At the global scale, many of the crenophilous invertebrates are stenothermal, which is related to their evolutionary origins (Sun et al. in press), and the stable thermal environments of spring-fed streams

may provide refugia for these coldwater taxa (Lusardi et al. 2016). The taxa that were dominant in the spring-fed tributary included stenothermal species, implying that unique assemblages may be found in such streams. This may be an important function of spring-fed streams; their unique habitats may contribute to the beta diversity of focal river networks.

We found a relatively homogenous streambed with high fine sediment deposition in the spring-fed tributary, yet taxa richness was highest in this reach. Lusardi et al. (2016) reported that macroinvertebrate density was higher but taxa richness was lower in volcanic spring-fed streams compared to run-off-fed streams. Because we sampled macroinvertebrates on a single occasion in June, our samples may not reflect all taxonomic groups. Ephemeroptera and Trichoptera species in particular may not have been captured well by June sampling based on their phenology, whereas the dominant chironomid taxa in the spring-fed tributary are multivoltine and thus were likely well reflected in our samples. Seasonal sampling in future studies would provide a more complete macroinvertebrate assessment.

Our comparisons of spring-fed and non-spring-fed streams revealed unique habitat characteristics and macroinvertebrate assemblage structure in the spring-fed tributary. The macroinvertebrate assemblage was characterized by numerous burrowers and collector-gatherers, and possibly crenobiont and/or crenophilous taxa. This unique assemblage may therefore enhance the beta-diversity of this river network. Owing to the high cover of fine sediments, the abundance of burrower taxa was remarkably high in the spring-fed tributary. These taxa may further play significant roles as prey for carnivores in the surrounding area. Given that spring-fed streams may act as flow and temperature refugia (Sakai et al. 2020, Lusardi et al. 2016), further studies that examine

the ecosystem functions of spring-fed streams can inform biodiversity conservation in river ecosystems. Here, we highlight the ecosystem function of providing unique habitats and housing unique macroinvertebrate assemblages.

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Table 1

Environmental characteristics of each study reach. Different alphabetical letters indicate statistically significant differences ($P < 0.05$).

	Tributary						Mainstream								
	ST1			ST2			ST3			ST4			ST5		
Variable	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Stream width (m)	3.81	±	0.30^{c}	0.99	±	0.16^{d}	10.35	±	0.22^{b}	15.33	±	1.34^{a}	12.30	±	1.65^{b}
Water depth (cm)	16.80	±	3.83^{d}	9.43	±	1.91^{e}	32.95	±	1.33^{b}	33.33	±	2.43^{a}	29.83	±	2.81^{c}
Current velocity (cm/s)	12.69	±	3.01^{d}	24.46	±	5.53^{c}	29.19	±	4.01^{bc}	36.91	±	8.25^{b}	59.45	±	13.78^{a}
Canopy openness (%)	34.32	±	12.11^{b}	45.46	±	17.38^{b}	48.88	±	15.32^{ab}	70.22	±	4.78^{a}	46.12	±	9.64^{b}
Fime sediment deposition (%)	83.50	±	10.55^{a}	22.00	±	7.15^{b}	8.00	±	3.50^{c}	8.00	±	4.83^{c}	6.00	±	2.11^{c}
Periphyton biomass (mg/cm^2)	1.43	±	0.38^{ab}	1.50	±	0.27^{ab}	1.68	±	0.00^{ab}	1.91	±	0.26^{a}	1.23	±	0.16^{b}
Standing stock of detritus (mg/cm^2)	1.65	±	0.88	0.72	±	1.03	0.28	±	0.24	0.21	±	0.10	0.33	±	0.17

Table 2

Total abundance, taxa richness, and the abundance of each dominant taxa in study reaches. LFT: life form type, B: burrower, Sp: sprawler, C: clinger, Sw: swimmer. FFG: functional feeding group, C/G: collector-gatherer, P: predator, G: grazer. Different letters indicate statistically significant differences ($P < 0.05$). Asterisks indicate that multiple comparisons were not performed.

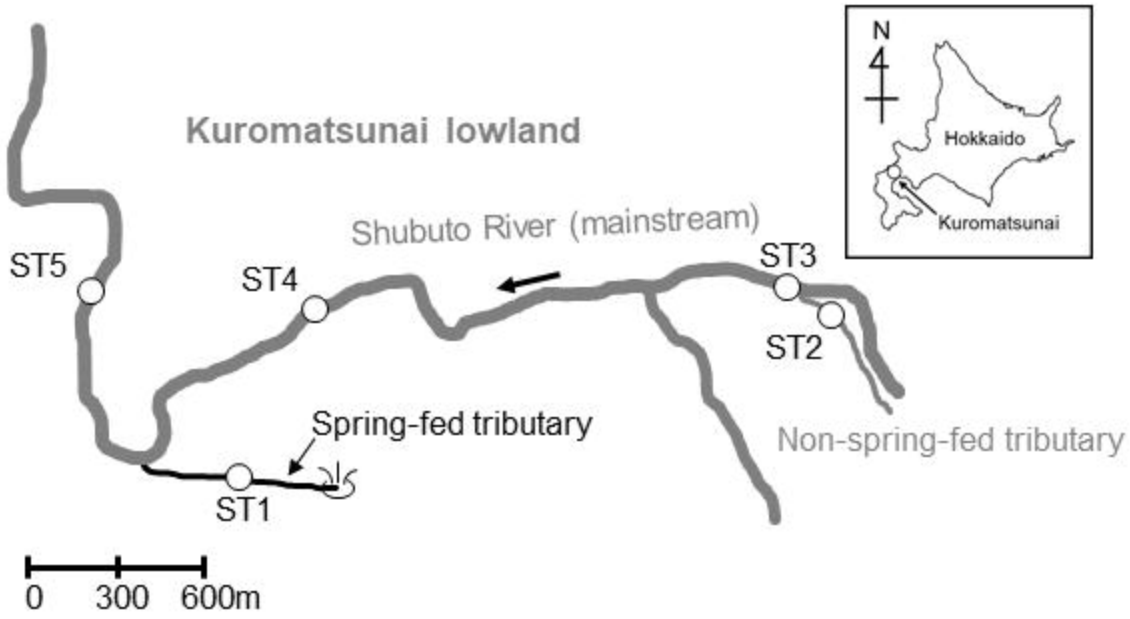
			Tributary						Mainstream								
			ST1			ST2			ST3			ST4			ST5		
Variable	LFT	FFG	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD
Total abundance	-	-	416.40	±	210.90[a]	34.00	±	18.21[c]	114.40	±	53.97[b]	87.60	±	47.06[bc]	123.40	±	63.03[b]
Taxa richness	-	-	23.80	±	3.83[a]	10.00	±	3.94[c]	19.40	±	4.04[ab]	16.60	±	2.70[bc]	17.60	±	2.70[ab]
Dominant taxon																	
Oligochaeta	B	C/G	116.60	±	141.46[a]	0.60	±	1.34[d]	14.00	±	6.89[bc]	2.40	±	1.82[cd]	44.60	±	53.48[ab]
**Pagastia* spp.	B	C/G	116.20	±	52.88	0	±	0	0	±	0	0.40	±	0.89	0.80	±	0.84
Drunella ishiyamana	Sp	P, G	2.60	±	2.07[ab]	0.20	±	0.45[b]	37.00	±	23.80[a]	31.20	±	17.25[a]	27.20	±	22.86[ab]
**Apatania* sp.	C	G	34.80	±	21.72	7.00	±	4.58	0.40	±	0.89	0	±	0	0	±	0
*Chironomidae gen. spp.	B	C/G	40.00	±	41.55	0	±	0	0	±	0	0	±	0	0	±	0
*Chironominae spp.	B	C/G	30.20	±	31.90	0	±	0	0	±	0	0	±	0	0	±	0
**Caenis* sp.	C	C/G, G	0	±	0	0	±	0	14.80	±	10.50	6.20	±	4.92	5.20	±	6.06
Baetis sp.	Sw	G	3.00	±	3.24	2.60	±	3.44	3.40	±	2.61	7.20	±	6.30	4.00	±	2.00
Dicranota spp.	B	P	5.40	±	2.61[a]	4.40	±	3.44[ab]	1.20	±	1.64[b]	5.60	±	4.56[a]	2.00	±	1.00[ab]
**Allomyia* sp.	C	G	18.40	±	11.70	0	±	0	0	±	0	0	±	0	0	±	0
Epeorus sp.	Sp	G	0.20	±	0.45[bc]	0	±	0[c]	11.60	±	6.23[a]	2.60	±	2.07[abc]	3.80	±	2.59[ab]

Ordobrevia sp.	Sp	C/G	1.80	±	1.30[ab]	4.20	±	1.30[a]	1.00	±	0.71[b]	7.80	±	9.18[a]	3.40	±	1.52[ab]
Ephemerella setigera	Sp	C/G	0.40	±	0.55[ab]	0	±	0[b]	2.20	±	1.92[ab]	5.20	±	3.11[ab]	9.40	±	9.91[a]
Ecdyonurus viridis	Sp	G	0.40	±	0.55[bc]	0	±	0[c]	3.60	±	2.79[ab]	0.40	±	0.55[bc]	7.80	±	4.92[a]
*Tanypodinae sp.	B	P	12.20	±	9.93	0	±	0	0	±	0	0	±	0	0	±	0
Drunella sp.	Sp	P, G	0.20	±	0.45[b]	0	±	0[b]	3.80	±	3.56[a]	5.40	±	3.65[a]	1.40	±	1.14[ab]
Orthocladiinae sp.	B	C/G	2.00	±	1.87	1.20	±	0.84	1.60	±	1.14	1.40	±	0.89	3.20	±	1.64
Paraleptophlebia japonica	B	C/G	2.40	±	3.71[ab]	0.60	±	1.34[ab]	3.20	±	1.30[a]	0.20	±	0.45[b]	1.60	±	1.67[ab]

Figure legends

Fig. 1 Study site location within the Shubuto River system in Kuromatsunai, Hokaido, Japan.

Fig. 1


Kuromatsunai lowland
Shubuto River (mainstream)
ST5
ST4
ST3
ST2
ST1
Spring-fed tributary
Non-spring-fed tributary
0 300 600m
N
Hokkaido
Kuromatsunai