NOTE AND COMMENT

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On the coexistence of three predatory stonefly species in a central Japanese stream

Abstract Three large-bodied stonefly species (*Paragnetina tinctipennis*, *Oyamia lugubris*, and *Kamimuria tibialis*) coexist in a central Japanese stream. These species have been classified as predators. Here we study their microhabitat use while focusing on the physical environments, physiological activity, and food resources. We show that *Paragnetina* uses a niche with faster currents than other species throughout the year. *Oyamia* has seasonal flexibility in microhabitat preference, physiological activity, and food resources. *Kamimuria* is a rather stable species, independent of seasonal patterns.

Keywords Coexistence · Current · Diet · Predators · Stonefly

Introduction

In a mountain stream of central Japan, three species of large-bodied stonefly, *P. tinctipennis* McLachlan, *O. lugubris* McLachlan and *K. tibialis* Pictet, occur together within a reach scale (Miyasaka and Genkai-Kato 2008). Large-bodied stoneflies have been regarded as predators in their late nymphal stages, but a recent study on food use of stream invertebrate predators using stable isotope analysis showed that many large-bodied stoneflies are omnivores consuming both animal and plant materials (Lancaster et al. 2005). In a mountain stream of central Japan, the relative dependence of the three predatory stoneflies on animal and plant food resources can change seasonally such that *Paragnetina* and *Oyamia* are car-

nivores in summer, whereas all species in winter and *Kamimuria* in summer consume both animal prey and algae (Miyasaka and Genkai-Kato 2008). *Paragnetina* and *Oyamia* overlap in their diets in summer, and diets are similar among all species in winter. Therefore, diet separation cannot necessarily explain why these species co-occur within a reach scale.

Uchida (1990) showed that current is an important physical factor influencing the spatial separation of stoneflies. One of the three species, *O. lugubris*, is a species sensitive to oxygen availability which is closely related to both current and dissolved oxygen concentration (Genkai-Kato et al. 2000). Oxygen conditions can be an important factor influencing the distribution of stoneflies within a reach scale, and, actually, the habitat preference of *Oyamia* for current changes seasonally (Genkai-Kato et al. 2005). Moreover, Isobe (1989) suggested temporal separation of life cycle and emergence period for coexistence among similar stonefly species. In this study, taking into consideration the diets, microhabitat preferences, and life cycles of three predatory stonefly species, we studied their coexistence mechanisms within a reach scale.

Methods

Field study was conducted in Kuro-kawa River (35°53′N, 137°40′E) in Kiso, Nagano Prefecture, Japan, in summer and winter during the daytime (1000–1700 hours). Kuro-kawa River (14 km long) is a mountain stream in central Japan, tributary to the Kiso River system flowing into the Pacific Ocean. A 100-m stretch of the stream, 9.5 km downstream from the headwater, was selected as the study reach for collecting organisms. The physical characteristics of the study reach were (mean ± SE): width, 10.8 ± 0.5 m in summer, $n = 25$ and 9.9 ± 0.3 m in winter, $n = 16$; water temperature, $18.2 \pm 0.8°C$ in summer, $n = 4$ and $3.6 \pm 0.1°C$ in winter, $n = 4$; altitude, 840 m; gradient, 2.6%.

We focused on nymphs of three dominant species of large-bodied stonefly (Perlidae), *Paragnetina tinctipen-*

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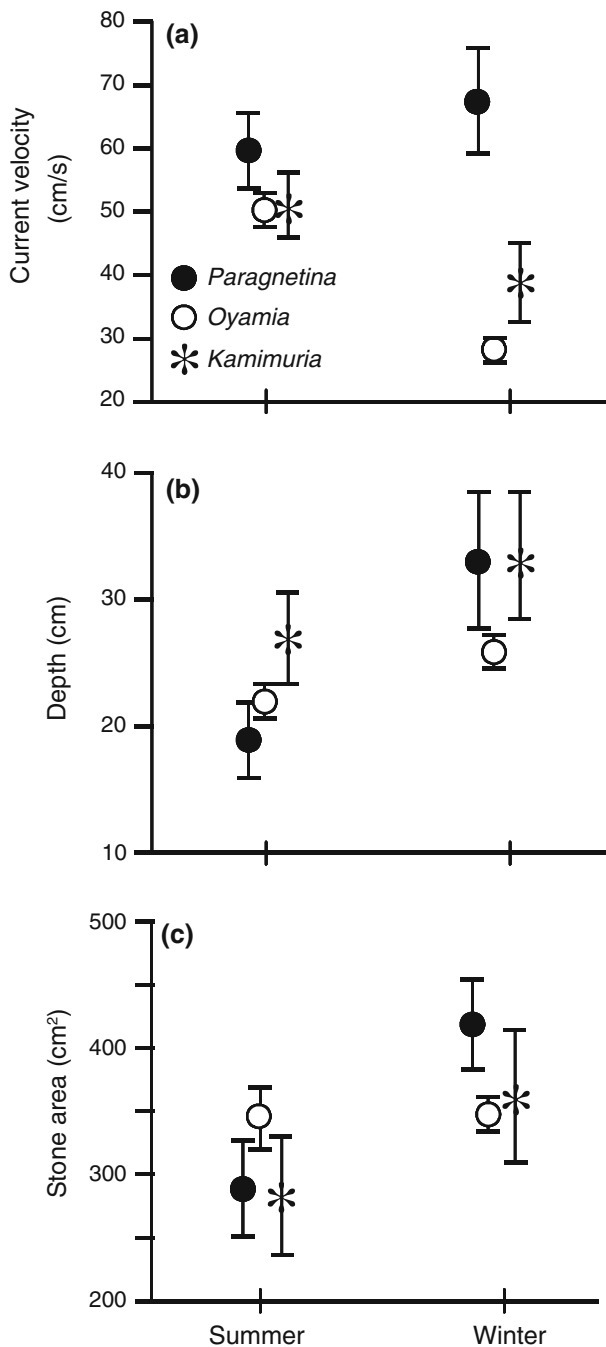Tel.: +81-77-5498200
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Table 1 Density (ind m^{-2}), proportional abundance (%, calculated based on dry weight), and total dry mass of aquatic invertebrates collected by a large Surber net sampler ($n = 5$ for each season, mean $\pm$ SE)

Taxon		Summer		Winter	
		Density	% dry mass	Density	% dry mass
Coleoptera					
Elmidae		0	0	3 $\pm$ 3	0.02 $\pm$ 0.02
Diptera					
Blephariceridae		0	0	2 $\pm$ 2	0.30 $\pm$ 0.22
Chironomidae		604 $\pm$ 74	2.97 $\pm$ 0.77	6,499 $\pm$ 1,158	39.95 $\pm$ 9.94
Simuliidae		46 $\pm$ 16	0.28 $\pm$ 0.08	23 $\pm$ 10	0.11 $\pm$ 0.04
Tipulidae	Limoniinae	14 $\pm$ 3	0.62 $\pm$ 0.35	38 $\pm$ 8	0.92 $\pm$ 0.49
	Tipulinae	6 $\pm$ 3	3.49 $\pm$ 3.00	9 $\pm$ 3	2.17 $\pm$ 1.04
Ephemeroptera					
Ameletidae	*Ameletus* spp.	22 $\pm$ 10	0.12 $\pm$ 0.08	166 $\pm$ 64	2.14 $\pm$ 0.39
Baetidae	*Baetis* spp.	894 $\pm$ 80	9.33 $\pm$ 1.93	249 $\pm$ 70	1.29 $\pm$ 0.29
Ephemerellidae	*Cincticostella* spp.	94 $\pm$ 19	1.65 $\pm$ 0.52	573 $\pm$ 88	6.26 $\pm$ 0.93
	Drunella spp.	158 $\pm$ 29	4.78 $\pm$ 0.60	66 $\pm$ 62	4.85 $\pm$ 4.12
Ephemeridae	*Ephemera japonica*	0	0	1 $\pm$ 1	0.01 $\pm$ 0.01
Heptageniidae	*Cinygmula* spp.	104 $\pm$ 15	3.78 $\pm$ 0.37	52 $\pm$ 7	1.41 $\pm$ 0.48
	Epeorus spp.	229 $\pm$ 63	3.78 $\pm$ 0.37	293 $\pm$ 174	1.41 $\pm$ 1.48
Leptophlebiidae	*Paraleptophlebia* spp.	34 $\pm$ 16	0.35 $\pm$ 0.16	4 $\pm$ 2	0.02 $\pm$ 0.01
Megaloptera					
Corydalidae	*Protohermes grandis*	6 $\pm$ 3	26.14 $\pm$ 11.72	10 $\pm$ 5	10.32 $\pm$ 5.04
Odonata					
Epiophlebiidae	*Epiophlebia superstes*	1 $\pm$ 1	0.22 $\pm$ 0.22	3 $\pm$ 2	0.60 $\pm$ 0.55
Plecoptera					
Leuctridae	*Paraleuctra* sp.	2 $\pm$ 2	0.01 $\pm$ 0.01	36 $\pm$ 8	0.57 $\pm$ 0.18
	Rhopalopsole sp.	2 $\pm$ 2	0.08 $\pm$ 0.08	9 $\pm$ 3	0.09 $\pm$ 0.05
Nemouridae	*Protonemura* sp.	8 $\pm$ 3	0.03 $\pm$ 0.01	8 $\pm$ 3	0.05 $\pm$ 0.04
Perlidae	*Calineuria* sp.	21 $\pm$ 6	1.16 $\pm$ 0.61	55 $\pm$ 13	0.54 $\pm$ 0.11
	Caroperla pacifica	2 $\pm$ 2	0.18 $\pm$ 0.18	2 $\pm$ 2	0.02 $\pm$ 0.02
	Kamimuria tibialis	3 $\pm$ 1	0.62 $\pm$ 0.32	3 $\pm$ 1	1.29 $\pm$ 0.84
	Kamimuria uenoi	3 $\pm$ 1	0.78 $\pm$ 0.54	2 $\pm$ 2	0.27 $\pm$ 0.27
	Oyamia lugubris	10 $\pm$ 6	7.83 $\pm$ 5.24	11 $\pm$ 5	6.36 $\pm$ 3.67
	Paragnetina tinctipennis	3 $\pm$ 2	10.32 $\pm$ 6.56	5 $\pm$ 1	2.06 $\pm$ 0.76
Perlodidae		0	0	2 $\pm$ 2	0.25 $\pm$ 0.25
Trichoptera					
Brachycentridae	*Micrasema* sp.	0	0	1 $\pm$ 1	0.04 $\pm$ 0.04
Glossosomatidae	*Glossosoma* sp.	42 $\pm$ 9	1.00 $\pm$ 0.48	286 $\pm$ 105	2.11 $\pm$ 0.80
Goeridae	*Goera* sp.	0	0	43 $\pm$ 24	3.57 $\pm$ 2.50
Hydropsychidae	*Hydropsyche orientalis*	51 $\pm$ 9	2.56 $\pm$ 0.64	18 $\pm$ 8	0.66 $\pm$ 0.35
Lepidostomatidae	*Lepidostoma* sp.	1 $\pm$ 1	0.01 $\pm$ 0.01	3 $\pm$ 2	0.05 $\pm$ 0.05
Limnephilidae		9 $\pm$ 7	0.32 $\pm$ 0.28	0	0
Rhyacophilidae	*Rhyacophila* spp.	19 $\pm$ 5	1.28 $\pm$ 0.66	38 $\pm$ 6	2.44 $\pm$ 0.38
Stenopsychidae	*Stenopsyche marmorata*	11 $\pm$ 8	0.48 $\pm$ 0.27	2 $\pm$ 2	4.21 $\pm$ 4.21
Hirudinea		0	0	1 $\pm$ 1	0.03 $\pm$ 0.03
Nematoda		194 $\pm$ 66	1.06 $\pm$ 0.75	84 $\pm$ 62	0.75 $\pm$ 0.43
Tricladida		7 $\pm$ 5	0.01 $\pm$ 0.01	1 $\pm$ 1	0.02 $\pm$ 0.02
Total dry mass (g)		3.09 $\pm$ 0.54		8.40 $\pm$ 1.98	

nis, *Oyamia lugubris*, and *Kamimuria tibialis*. Surveys of stonefly microhabitat were conducted in summer (five days: 26 July, 9–11 August, and 6 September 1999) and in winter (3 days: 11–13 January 1999). We established a 1 × 1 m grid over the study reach. Stonefly sampling was conducted in principle by collecting a stone (loose cobble) at each grid node. A hand net (27 × 27 cm, 1.9 mm mesh) was placed downstream of the stone, and then the whole stone was collected into the hand net along with its substrata (pebbles and sand). Sampling was conducted in a downstream to upstream manner so released nymphs would not be resampled. When a nymph of either *P. tinctipennis*, *O. lugubris* or *K. tibialis* was found in the net, we recorded species of the nymph and then

released it. At the same time, we measured current velocity, depth, and the surface area of the stone where the nymph was collected. Current velocity was measured at 60% of the water column depth to estimate mean column velocity (Hynes 1970), using a portable current meter (Model CR-7WP, Cosmo-Riken, Kashihara). Surface area of the stone was approximated as an elliptic shape: $\pi/4 \times$ major axis $\times$ minor axis of the stone.
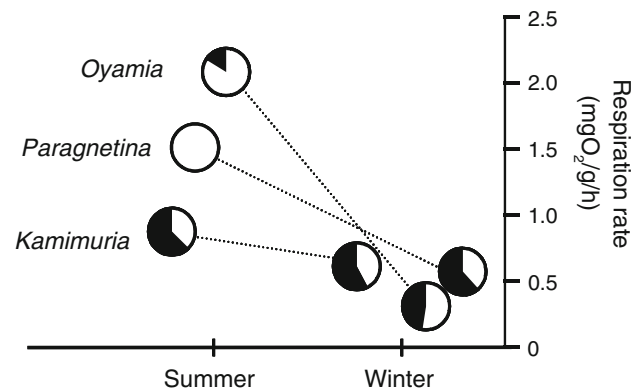
To estimate the abundance of the stoneflies among the invertebrate community in the stream, aquatic insects were collected with a large Surber net sampler (50 × 50 cm, 225-μm mesh) in summer (21 July 2001) and in winter (19 February 2001) in the study area ($n = 5$ in both seasons). Dry biomasses of the stoneflies

Fig. 1 Current velocity (a), depth (b), and stone area (c) at microhabitats of three stonefly species in summer and winter (mean ± SE)

and other smaller invertebrates were estimated according to Genkai-Kato and Miyasaka (2007) and Miyasaka and Genkai-Kato (2008), respectively.

In addition to the specimens collected in this study, we used our accumulated data on head capsule width of the stoneflies since 1997 in the stream, because head capsule widths were often used to show the size distributions of stonefly nymphs (e.g., Isobe 1989; Lancaster et al. 2005; Miyasaka et al. 2008). For head capsule width measurement, live stonefly nymphs were transported in cool water

Fig. 2 Respiration rates (y-axis) and food resources (circles) of three stonefly species measured in summer and winter (modified after Miyasaka and Genkai-Kato 2008). Circles indicate the relative dependence on animal and plant materials as food resources in whole tissues of the stoneflies determined by $\delta^{15}N$ stable isotope analysis. Open and closed areas in circles correspond to animal and algal foods, respectively

to the laboratory within 3 h after collection. Individual nymphs, which were placed in a transparent plastic bag (Unipack, A-4: $70 \times 50 \times 0.04$ mm, Seinichi, Tokyo) to reduce movement of the nymph due to the slippery surface of the bag, were measured for head capsule width and body length to the nearest 0.1 mm using a digital caliper (Digimatic Caliper, Series No. 500, Mitsutoyo, Kawasaki).
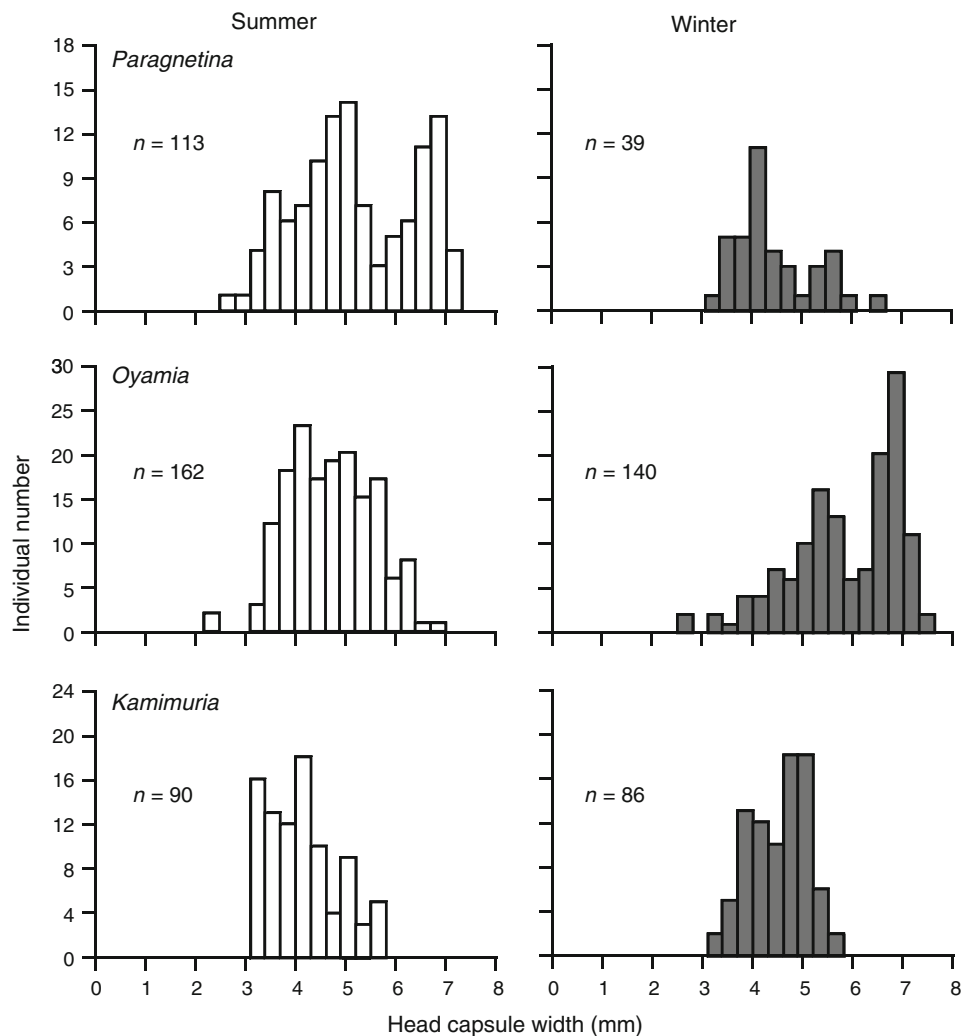
Results and discussion

Neither current velocity ($F_{1,395} = 2.84$, $P = 0.093$; mean ± SE = 36.4 ± 1.9 cm s^{-1}, $n = 245$ in summer; 31.4 ± 2.2 cm s^{-1}, $n = 152$ in winter) nor depth ($F_{1,395} = 1.20$, $P = 0.273$; 32.3 ± 2.0 cm, $n = 245$ in summer; 29.2 ± 1.5 cm, $n = 152$ in winter) at grid nodes differed significantly between seasons (by one-way ANOVA). However, there was a significant difference in stone area between seasons ($F_{1,395} = 7.48$, $P = 0.007$; 243 ± 10 cm^2, $n = 245$ in summer; 284 ± 11 cm^2, $n = 152$ in winter).

Listed in Table 1 are the density (ind m^{-2}) and proportional abundance expressed by dry weight (%) of the aquatic invertebrate community (including the predatory stoneflies and their potential prey) in the study reach. The three species, P. tinctipennis, O. lugubris and K. tibialis, accounted for 18.8 and 9.7% in dry mass of the total invertebrate community in summer and in winter, respectively. These species were dominant stoneflies and accounted for more than 80% of the total plecopteran biomass throughout the year.

From the microhabitat surveys, we collected 19 (8), 102 (87), and 27 (15) individuals of Paragnetina, Oyamia, and Kamimuria, respectively, in summer (in winter). Shown in Fig. 1a is the relationship of microhabitat of these species to current velocity. Two-way ANOVA revealed that seasonal change in current

Fig. 3 Frequency distribution of head capsule width of three stonefly species in summer and winter. Total sample numbers for each species in each season are indicated (n)

velocity at stonefly microhabitats differed significantly between species (species, $F_{2,252} = 9.76$, $P < 0.001$; season, $F_{1,252} = 3.66$, $P = 0.057$; interaction, $F_{2,252} = 3.89$, $P = 0.022$). *Paragnetina* preferred sites with fast current (Fig. 1a). The current velocity at *Oyamia* microhabitats in summer was faster than that in winter. *Kamimuria* stayed at intermediate to slow current sites throughout the year.

Two-way ANOVA revealed that depth of stonefly microhabitats did not differ between species, but there was a significant difference in depth between seasons (species, $F_{2,252} = 2.62$, $P = 0.075$; season, $F_{1,252} = 8.30$, $P = 0.004$; interaction, $F_{2,252} = 1.11$, $P = 0.333$; Fig. 1b). The presence of stonefly nymphs did not depend on stone area for any species or either season (species, $F_{2,252} = 0.21$, $P = 0.811$; season, $F_{1,252} = 3.37$, $P = 0.068$; interaction, $F_{2,252} = 1.35$, $P = 0.262$; Fig. 1c).

These results showed that current was one of the dominant physical factors determining microhabitats of the stoneflies, in agreement with Uchida (1990). *Paragnetina* and *Kamimuria* preferred fast and slow-current sites, respectively, regardless of season. *Oyamia* dis-

played a seasonal shift between these sites. Miyasaka and Genkai-Kato (2008) reported that there were species-specific seasonal patterns in physiological activity and in diet between these species. The physiological activities of *Paragnetina* and *Oyamia* in winter were significantly reduced from those in summer, while *Kamimuria* maintained a constant physiological activity throughout the year (Fig. 2). Interestingly, there was positive correlation between physiological activity and carnivory such that, regardless of species, individuals with high physiological activity prey mainly on animals, while those with low physiological activity ingested both animal and plant materials. High physiological activity associated with carnivory in summer may force stoneflies to stay at fast-current sites where oxygen supply is high due to high water-renewal rates, because otherwise they are compelled to undertake respiratory behavior (i.e., push-ups; Genkai-Kato et al. 2000). Behavior such as doing push-ups can be costly for predatory stoneflies because it cannot be carried out simultaneously with other activities such as foraging.

The low oxygen demand of *Oyamia* in winter may be because of their preference for nearshore habitats with

slow currents in preparation for emergence in spring (Maruyama and Takai 2000). The reasons for *Paragnetina* staying at fast-current sites in winter despite its reduced physiological activity could be the life history of, and competition between, predatory stonefly species. The distribution of stonefly size differed between species (Fig. 3). Remarkably large individuals of *Paragnetina* appeared in summer, while those of *Oyamia* appeared in winter. *Kamimuria* nymphs did not reach such large sizes. Our seasonal distribution patterns of head capsule width in Fig. 3 suggest that the emergence of *Paragnetina* and *Oyamia* occurs after and before the summer, respectively. Generally in central Japan, *P. tinctipennis* has a two or three-year life cycle and emerges in late spring to summer, and *O. lugubris* has a two or three-year life cycle and emerges during spring (Maruyama and Takai 2000). Our size-distribution data partly conform to the emergence pattern in central Japan—*Oyamia* emerges earlier than *Paragnetina*. A longer life cycle and later emergence can occur in cold waters (Kawai and Tanida 2005), which may be the case with *Paragnetina* in a high-altitude stream (3.6–18.2°C and 840 m above sea level). In winter, *Paragnetina* may avoid slow-current sites where larger individuals of *Oyamia* are present to minimize resource competition, because the food dependences of both species are similar (i.e., approximately 50% carnivory and 50% algivory, Fig. 2). Because *Oyamia* is the most dominant species in abundance and size in winter among predatory stoneflies (Table 1, Fig. 3), the competitive influence of *Oyamia* on other species is likely to be greater than reversed influences. In contrast, in summer, larger individuals of *Paragnetina* may overcome the competitive influence of *Oyamia* coexisting in fast-current sites. A slight dietary dependence of *Oyamia* on plant materials (21% algivory) despite high physiological activity in summer may reflect a competitive advantage of *Paragnetina* (100% carnivory) over *Oyamia* in fast-current sites (Fig. 2).

In winter, *Kamimuria* shared slow-current habitats with *Oyamia* (Fig. 1a). *Kamimuria* displayed considerably different characteristics from the other species in terms of seasonal changes in current preference (Fig. 1 a), diet, physiological activity (Fig. 2), and body size (Fig. 3). These characteristics of *Kamimuria* are seasonally stable compared with those of *Paragnetina* and *Oyamia*, rather independent of season. Our results suggest that the niche of *Kamimuria* may be different from those of *Paragnetina* and *Oyamia*. However, more detailed research with regard to their niche characteristics should be explored as a future study to clarify the coexistence mechanisms between the predatory stonefly species within a reach scale.

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