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ORIGINAL ARTICLE

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Shift between carnivory and omnivory in stream stonefly predators

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Abstract Omnivory can have profound effects on the trophic dynamics of communities and ecosystems, as they may interact with multiple trophic levels simultaneously. Some species of large-bodied stoneflies may be viewed as omnivores rather than true carnivores even at later nymphal stages. We evaluated the seasonal change in the diet of stonefly predators by analyzing their stable isotope ratio, gut contents, physiological activity, and food availability. A two-source-based mixing model based on stable isotope analysis revealed that stoneflies shifted their diet between carnivory in summer and omnivory in winter—despite the higher availability of animal prey in winter. The gut content analysis showed that swift prey (mayflies) were consumed in the summer, whereas sluggish prey (Chironomidae) were consumed in the winter. The physiological activity of stoneflies also declined markedly in winter. These results suggest that, in winter, stoneflies foraged on a mixture of Chironomidae and algae. It appears that omnivory in some stream consumers is related to the seasonal change in temperature-dependent physiological activity, rather than prey availability.

Keywords Omnivory · Physiology · Predator · Stonefly · Water temperature

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Introduction

Diet switching among food items by predators is a common strategy to maximize predators' energy gain and is predicted to play an important role in the maintenance of food-web stability (Pimm and Lawton 1978; McCann et al. 1998; Vadeboncoeur et al. 2005). Diet shifts among multiple trophic levels (omnivory), particularly between autotrophs and heterotrophs, require changes in physiological (Buchholz and Saborowski 2000) and morphological specialization (Herrel et al. 2004) compared to shifts among food items within a trophic level. Animals have been often observed to display ontogenetic shifts in diet (Allan 1982; Feminella and Stewart 1986). This trend from herbivory to carnivory, with temporary omnivory in between, is a reasonable step in terms of evolutionary success because efficiently ingestible prey (i.e., animals) become more available with the growth stages of the predators due to the increased ability of the latter to capture prey (Werner and Gilliam 1984).

In theory, diet choice is determined by the density of the preferred food items (Charnov 1976). However, this feeding theory fails to consider the physiological capacity of a predator to be active and capture the preferred prey. If the feeding activity is independent of a predator's physiological condition, each prey item would be consumed in similar proportions in the summer and winter. In poikilotherms, metabolic and, consequently, behavioral activities are highly dependent on the surrounding temperature (Taniguchi and Nakano 2000). If activity is low due to low temperature, animals may have difficulty in capturing prey that they would otherwise prey on at higher temperatures. Under low temperature conditions, animals may subsist by consuming suboptimal food items that are easily obtained with a lower energy expenditure if predators and prey have different temperature optima for physiological activity. Since the consumption of sessile plants and animals is accompanied by less energy expenditure compared with the

pursuit and subsequent attack of mobile animals, carnivores may change their ecological position to omnivory or herbivory under conditions of lower temperature.

Perlidae stonefly species are viewed as carnivores during the late stages of nymphal development, but some plant material is often found in their diet (e.g., Hynes 1970). Some large-bodied stoneflies may be viewed as omnivores rather than true carnivores even at later nymphal stages (Lancaster et al. 2005). However, the mechanism which causes carnivorous stoneflies to consume plant materials is as yet unknown, as is whether this dependence on algae as food resources varies seasonally? Since water temperature depends primarily on the season, we have evaluated the seasonal change in food items of three stonefly species that are widespread in the mountain streams of central Japan by analyzing their gut contents, food availability and physiological activity. Stable isotope analysis of nitrogen was conducted to determine the food resources assimilated into stonefly tissues. We have also examined the possibility that carnivorous stoneflies shift from carnivory (i.e., insectivory) to omnivory (i.e., insectivory and algivory) under low temperature conditions.

Methods

Study area

The field study was conducted in the Kuro-kawa River (35°53′N, 137°40′E) area in Kiso, Nagano Prefecture, central Japan, during the daytime (1000–1700 hours), in the summer (20 July–04 August) and winter (17–25 December) of 2001. The Kuro-kawa River (14 km long) is a third-order mountain stream and a tributary to the Kiso River system flowing into the Pacific Ocean. Kuro-kawa means black river, and the name derives from its high algal productivity (thick, black algal mats on streambed stones) throughout the year despite the clear water. A 3.5-km stretch (6–12 m wide, 15–40 cm deep, 960–840 m a.s.l., 2.6–3.4% gradient) of the stream, 6.0–9.5 km downstream from the headwater, was selected as the study area for collecting and incubating organisms. There were clear differences in mean daytime water temperature (mean ± SE, 18.7 ± 0.3°C in summer, $n = 34$; 2.0 ± 0.3°C in winter, $n = 24$) and dissolved oxygen concentration (DO: 7.72 ± 0.05 mg O_2 L^{-1} in summer, $n = 23$; 12.15 ± 0.05 mg O_2 L^{-1} in winter, $n = 9$) between the seasons.

Study animals

We focused on three species of stonefly nymphs (Perlidae)—*Oyamia lugubris* McLachlan, *Paragnetina tinctipennis* McLachlan, and *Kamimuria tibialis* Pictet—as these were the dominant predatory stoneflies of the stream in terms of abundance. In this stream, *Oyamia* has a 2- or 3-year life cycle and emerges during the spring; *Paragnetina* has a 2- or 3-year life cycle and emerges during the summer to autumn; *Kamimuria* has a 1- or 2-year life cycle and emerges during the spring (Genkai-Kato and Miyasaka, submitted). To estimate their individual densities per unit area, we collected stoneflies with a quadrat net (20 × 20 cm, 3-mm mesh) from riffle reaches in the study area ($n = 245$ in summer, $n = 135$ in winter). A sub-sample of the collected nymphs were transported freshly to the laboratory for individual dry weight measurements (see Appendix 1 for sample numbers), and the other nymphs were released. Reaches were sampled in a downstream to upstream manner so released nymphs would not be resampled. The reaches used for estimating stonefly densities were separated from other sampling reaches used in the isotope, diet and physiological activity analyses. Stonefly samples transported to the laboratory were dried at 60°C for 48 h, cooled in a desiccator, and weighed to the nearest 0.1 mg using an electric balance (AB135-S; Mettler Toledo, Greifensee, Switzerland).

Invertebrate prey were collected using a large Surber net sampler (50 × 50 cm, 225-μm mesh) from riffle habitats in the study area ($n = 5$ in both seasons). Based on our preliminary gut content analysis of these stonefly species, we focused on *Baetis*, *Epeorus* (Ephemeroptera), and Chironomidae (Diptera) as the main animal-based diet (defined as "animal prey") for the stoneflies in this study. Collected invertebrate prey were enumerated using a binocular dissecting microscope. Counts of individual prey were converted to taxon-specific mean dry mass using the individual dry weight for each season listed in Appendix 2, and prey biomass were expressed as dry mass per unit area (mg m^{-2}).

To estimate the abundance of benthic algae (plant-based diet, defined as "algae"), biofilm was removed from the upper surface (5 × 5 cm in area) of a stone using a toothbrush ($n = 9$ in summer, $n = 10$ in winter). The biofilm mixed with stream water was collected on a Whatman GF/C glass-fiber filter. The filter was then put into a 90% acetone solution to extract chlorophyll *a* for 24 h under a dark, cool condition. The amount of extracted chlorophyll *a* was measured by the Unesco method (Unesco 1966) using a spectrometer (U-1100; Hitachi, Tokyo, Japan). Algal abundance was expressed as chlorophyll *a* per unit area (mg chl *a* m^{-2}).

Stable isotope analysis

To evaluate the extent to which gut contents were assimilated into stonefly tissues, the three stonefly species and their potential food organisms (*Baetis, Epeorus*, and Chironomidae) were collected for stable isotope analysis in the study area. Stonefly nymphs (see Appendix 1 for sample numbers) and animal prey ($n = 10$ for each taxon in each season) were collected

with a normal Surber net sampler (25 × 25 cm, 225-μm mesh) from the study area. We measured the head capsule widths of stonefly nymphs as an indicator of body size (e.g., Lancaster et al. 2005) to the nearest 0.1 mm using a digital caliper (Digimatic Caliper, series no. 500; Mitsutoyo, Kawasaki, Japan). Collected stoneflies and prey were starved for 48 and 24 h, respectively, and all specimens were frozen at −20°C until later analysis. All animal samples were dried at 60°C for 48 h, cooled in a desiccator, weighed to the nearest 0.1 mg using the electric balance, and then ground to fine powder.

The stable isotope ratio of nitrogen was measured using a mass-spectrometer (ANCA-SL; PDZ Europa/Sercon, Crewe, UK). The ratio was expressed by δ notation as parts per thousand (‰) differences from the standard: $\delta^{15}\mathrm{N} = (R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1) \times 1000$, where R = atom%(^{15}N)/atom%(^{14}N). Atmospheric nitrogen was used for the standard ratio for nitrogen. Analytical precision was better than ±0.01‰ (n = 5). L-histidine ($\delta^{15}\mathrm{N} = -7.81$‰) was used as the reference material.

The δ^{15}N value of benthic algae, which were assumed to be the main food resource for grazers, was estimated at 2.5‰ lower than the mean δ^{15}N value of the three dominant prey (*Baetis*, *Epeorus*, and Chironomidae) based on the theory of trophic enrichment between primary producers and grazers (Vander Zanden and Rasmussen 2001). We preliminary collected biofilm on stones in the study area and measured its stable isotope signature (n = 5), but the δ^{15}N value of the biofilm was higher than the expected value based on the stable isotopic enrichment theory (i.e., 2.5‰ difference between primary producers and their consumers): the mean δ^{15}N of biofilm was 0.39‰ higher in the summer and 1.5‰ lower in the winter than the mean δ^{15}N of animal prey. Based on this discrepancy between stable isotopic enrichment theory and the δ^{15}N of biofilm, we deduced that biofilm attached on stones was different from the main food resource for grazers (benthic algae), which we were unable to specify isotopically in this study. It has often been reported that biofilm collected from stones has a higher δ^{15}N value than grazers (e.g., Vander Zanden and Rasmussen 1999). To resolve the discrepancy in terms of the trophic enrichment of stable isotope signature, the baseline (i.e., benthic algae) in our stream ecosystem was deduced from the mean nitrogen fractionation between primary producers and grazers estimated by a literature review (Vander Zanden and Rasmussen 2001). It should be acknowledged here that the δ^{15}N fractionation factors for the plant–herbivore interface are known to have a large error variance (Vander Zanden and Rasmussen 2001). However, these authors also report that the error variance tends to be minor—provided that herbivores are used as the baseline trophic level. Following this suggestion, the algal δ^{15}N value was deduced from herbivores in this study.

A two-source-based mixing model based on the nitrogen isotopic values was used to determine the proportional contribution of animal prey and algae assimilated into the stonefly tissues (Vander Zanden and Rasmussen 2001). The δ^{15}N value of stonefly (N_{predator}) that is dependent on animal prey (proportion of p) and algae ($1-p$) as food resources is given by the following equation:

$$N_{\mathrm{predator}} = p \cdot \left(N_{\mathrm{prey}} + 3.4\right) + (1-p) \cdot \left(N_{\mathrm{algae}} + 2.5\right), \tag{1}$$

where N_{prey} and N_{algae} are the δ^{15}N values of animal prey and algae, respectively. We have assumed that predation caused +3.4‰ trophic enrichment of δ^{15}N for carnivory and +2.5‰ for herbivory (Vander Zanden and Rasmussen 2001). The N_{prey} was the mean δ^{15}N value of *Baetis*, *Epeorus*, and Chironomidae. Rewriting Eq. 1, we have the proportional contribution of animal prey:

$$p = \frac{N_{\mathrm{predator}} - N_{\mathrm{algae}} - 2.5}{N_{\mathrm{prey}} - N_{\mathrm{algae}} + 0.9}. \tag{2}$$

Diet analyses

To evaluate the diet of the stoneflies, we collected stonefly nymphs from the study area with a normal Surber net sampler (see Appendix 1 for sample numbers) and placed them into a freezer as quickly as possible. We conducted two different analyses of their gut contents: direct observation for animal prey and chlorophyll *a* extraction from gut contents for algae. The gut, including gut contents, of an unfrozen stonefly nymph was carefully taken out of the body with forceps and placed on a filter paper. Animal prey items in the gut were identified to the lowest taxonomic level possible and enumerated under a dissecting microscope. Counts of individual prey were converted to dry mass of each taxon, based on the taxon-specific individual mean dry weight (Appendix 2). The amount of animal prey in the gut was expressed as the dry mass of each animal prey per unit dry mass of a stonefly nymph. After the removal of prey from the paper filter, the filter stained by gut contents, including chlorophyll *a*, was put into a 90% acetone solution for 24 h under a dark, cool condition. The amount of algae in the gut was measured as chlorophyll *a* using the spectrometer (Miyasaka and Genkai-Kato 2007) and expressed as chlorophyll *a* per unit dry mass of a stonefly nymph.

Physiological activity measurements

Physiological activity was measured as the respiration rate of invertebrates incubated in a DO bottle. Stonefly nymphs and animal prey were collected with a normal Surber net sampler from the study area. Incubation bottles (0.3 L) were placed on the streambed in the study area and covered by a black plastic sheet to mimic light conditions under a stone in their natural environment. A

stainless steel net (15 × 40 mm, 1.7-mm mesh) was placed in the incubation bottle with stonefly nymphs to reduce movement that would cause any unnatural additional consumption of oxygen (Genkai-Kato et al. 2000). After incubation, a portion of the water in the 0.3-L DO bottle was carefully transferred into a 0.1-L DO bottle using a silicon tube (8 mm in diameter) for the determination of DO by the Winkler method. Bottles with ambient stream water (control) were treated using the same protocol as that for the bottles with insects in terms of incubation time and transfer from 0.3-L bottles into 0.1-L bottles. A stainless steel net was also added into the control bottles for the determination of respiration rate by stonefly nymphs. Temperature-dependent metabolism was taken into account; therefore, the incubation time was 3 h (6 h in winter) for stonefly nymphs and 8 h (24 h in winter) for animal prey in the summer. The individual number of organisms in a DO bottle varied: for stoneflies, there was one stonefly per bottle (see Appendix 1 for replications, n); for *Baetis*, 15 in summer ($n = 4$) and ten in winter ($n = 6$); for *Epeorus*, five in both seasons ($n = 6$ in summer, $n = 7$ in winter); for Chironomidae, 30 in summer ($n = 7$) and ten in winter ($n = 6$). After incubation, the body length of stonefly nymphs was measured to obtain their dry mass using length–weight relationships (Genkai-Kato and Miyasaka 2007). The dry mass of animal prey was determined based on the taxon-specific individual mean dry weight (Appendix 2).

Respiration rate (RR, mg O_2 g^{-1} h^{-1}) was calculated from the difference in DO between the control bottle with ambient stream water and the bottle with insect(s). The respiration rate is expressed as the weight-specific value:

$$\mathrm{RR} = \frac{\mathrm{DO}_c \cdot V - \mathrm{DO}_I \cdot (V - V_{\mathrm{insect}})}{W \cdot m \cdot t}, \quad (3)$$

where V is the volume of the DO bottle for incubation (0.3 L); V_{insect} is the total volume of incubated insect(s); DO_c and DO_I are the dissolved oxygen concentrations (mg O_2 L^{-1}) in the control and bottle with insect(s), respectively, determined using a 0.1-L DO bottle; W is the individual mean dry mass (g); m is the number of individuals per incubated bottle; t is the incubation time (h). The total wet mass of incubated insects was converted to their total volume assuming that the relative density of insects was equal to water. We neglected the volume of stainless steel net ($< 4 \times 10^{-5}$ L).

Statistical analyses

Two-way ANOVAs were performed on the dry mass, density, chlorophyll *a* amount in stonefly gut, and animal prey amount in the stonefly gut as dependent variables, with stonefly species (*Oyamia*, *Paragnetina*, *Kamimuria*) and season (summer, winter) as factors. The effects of prey taxon (*Baetis*, *Epeorus*, Chironomidae) and season (summer, winter) as factors on the density of animal prey as a dependent variable were examined using a two-way ANOVA. A one-way MANOVA was used for comparing the diet in terms of animal prey composition in the gut among stonefly species. All statistical tests were two-tailed. Log_{10} transformations for exact values and arcsine-square-root transformations for percentages were conducted in order to standardize variances and improve normality. For any ANOVA model, multiple comparisons using the Scheffé test were conducted after one-way ANOVAs when necessary. An α level of 0.05 was set. Reduced α levels ($P = 0.05/n$ where n is the number of pairwise comparisons) were employed to correct for experiment-wise error in one-way MANOVAs.

Results

Individual numbers of stoneflies for each analysis are listed in Table 1. We dealt only with large-bodied individuals at later nymphal stages [head capsule width: *Oyamia* (mean ± SE), 4.71 ± 0.07 mm in summer and 5.85 ± 0.09 in winter; *Paragnetina*, 5.21 ± 0.11 in summer and 4.45 ± 0.12 in winter; *Kamimuria*, 4.13 ± 0.07 in summer and 4.52 ± 0.06 in winter]. Head capsule width data were collected from all stonefly individuals for individual dry weight, stable isotope, diet and physiological activity analyses (see Appendix 1). The seasonal change in dry body mass of individual stoneflies differed significantly between species (two-way ANOVA: species, $F_{2,199} = 10.55$, $P < 0.001$; season, $F_{1,199} = 0.57$, $P = 0.452$; interaction, $F_{2,199} = 29.01$, $P < 0.001$). The individual body mass of *Oyamia* was significantly greater in the winter (82 ± 7 mg) than in the summer (30 ± 2 mg; $F_{1,101} = 58.49$, $P < 0.001$). In contrast, the individual body mass of *Paragnetina* was lower in the winter (40 ± 4 mg) than in the summer (89 ± 11 mg; $F_{1,51} = 9.30$, $P = 0.004$). The individual body mass of *Kamimuria* was greater in the winter (36 ± 3 mg) than in the summer (26 ± 3 mg; $F_{1,47} = 4.30$, $P = 0.044$).

Table 1 δ^{15}N values of stoneflies, animal prey, and algae

Type	Taxon	Season	δ^{15}N value (‰) (mean ± SD)
Stonefly	*Oyamia*	Summer	5.25 ± 0.34
		Winter	5.89 ± 0.55
	Paragnetina	Summer	6.47 ± 0.55
		Winter	5.40 ± 0.22
	Kamimuria	Summer	3.80 ± 1.07
		Winter	5.60 ± 0.30
Animal prey	*Baetis*	Summer	2.71 ± 0.46
		Winter	4.23 ± 0.36
	Epeorus	Summer	2.83 ± 0.67
		Winter	4.61 ± 0.53
	Chironomidae	Summer	2.11 ± 0.31
		Winter	3.44 ± 0.52
Benthic algae		Summer	−0.29[a]
		Winter	1.23[a]

[a] 2.5‰ lower than the mean δ^{15}N value of three dominant prey (*Baetis*, *Epeorus*, and Chironomidae)

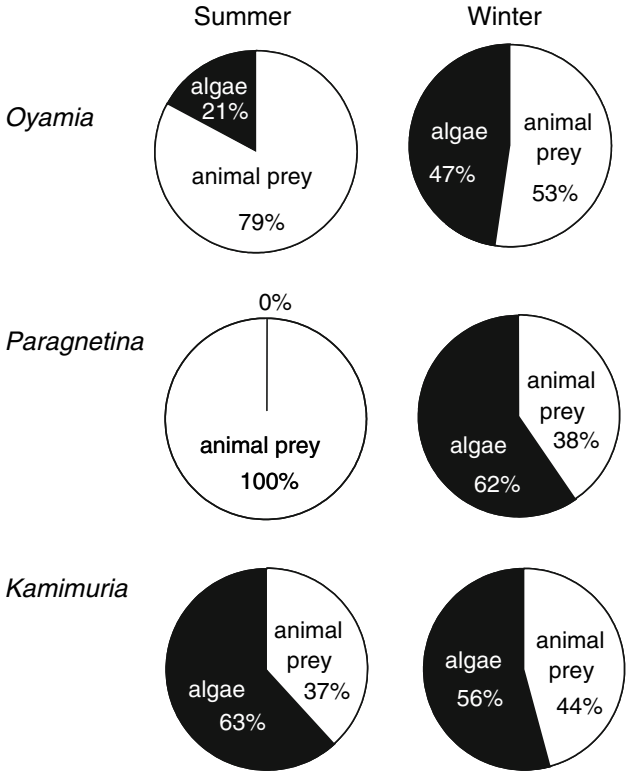

Fig. 1 Relative contribution of animal and plant material in whole-body tissues of three stonefly species determined by a mixing model based on the mean δ^{15}N values. *Open and filled areas in circles* Animal and algal foods, respectively. See Appendix 1 for sample numbers

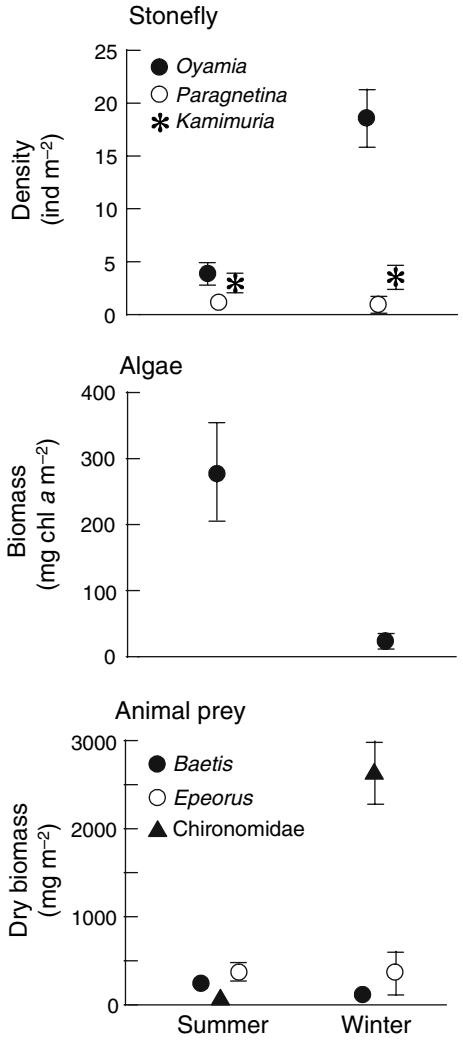

Fig. 2 Density of stoneflies and biomass of algae and animal prey measured in the summer and winter in the stream (mean ± SE)

Stable isotope analysis, based on the δ^{15}N results of organisms summarized in Table 1, revealed two types of diet patterns (Fig. 1). There was an isotopic diet shift for *Oyamia* and *Paragnetina* between seasons, while no seasonal isotopic diet shift was found for *Kamimuria*. Our mixing model suggested that, isotopically, the main food resources of *Oyamia* and *Paragnetina* were a mixture of animal prey in summer, indicating that they were carnivorous. In contrast, the same model suggested a mix of animal prey and algae, indicating omnivory in winter. *Kamimuria* was estimated to feed on both animal prey and algae and was omnivorous throughout the year.

Stonefly density differed significantly both among stonefly species and between seasons, with a significant interaction effect (two-way ANOVA: species, $F_{2,1134} = 40.37$, $P < 0.001$; season, $F_{1,1134} = 25.82$, $P < 0.001$; interaction, $F_{2,1134} = 24.25$, $P < 0.001$; Fig. 2). *Oyamia* density was 4.6-fold greater in winter (one-way ANOVA $F_{1,378} = 35.13$, $P < 0.001$), whereas *Paragnetina* and *Kamimuria* did not differ between seasons ($P > 0.706$ for both species). The abundance of benthic algae was 13-fold higher in the summer than in the winter (one-way ANOVA $F_{1,17} = 20.06$, $P < 0.001$; Fig. 2). The animal prey community was dominated by *Baetis*, *Epeorus*, and Chironomidae in both seasons. In terms of total abundance, measured as numbers of individual (or biomass) of benthic invertebrates (mean ± SE), these taxa accounted for 66.3 ± 3.4% (or 27.4 ± 2.7%) of the abundance in the summer and 81.0 ± 4.1% (or 45.2 ± 8.4%) in the winter. The seasonal change in abundance of animal prey differed significantly among three prey taxa (two-way ANOVA: prey, $F_{2,24} = 27.41$, $P < 0.001$; season, $F_{1,24} = 34.76$, $P < 0.001$; interaction, $F_{2,24} = 41.30$, $P < 0.001$; Fig. 2). Chironomidae abundance was 29-fold higher in the winter (one-way ANOVA $F_{1,8} = 80.23$, $P < 0.001$). In contrast, *Baetis* abundance was threefold higher in the summer ($F_{1,8} = 24.44$, $P = 0.001$). *Epeorus* did not differ in abundance between seasons ($P = 0.944$).

The seasonal change in chlorophyll *a* (an indicator of algal biomass) amount in the stonefly guts differed significantly between species (two-way ANOVA: species, $F_{2,113} = 8.55$, $P < 0.001$; season, $F_{1,113} = 24.11$, $P < 0.001$; interaction, $F_{2,113} = 12.84$, $P < 0.001$; Fig. 3a). *Oyamia* and *Kamimuria* had 4.7- and 1.6-fold,

respectively, greater amounts of algae in the gut in the winter than in the summer (*Oyamia*, $F_{1,38} = 23.46$, $P < 0.001$; *Kamimuria*, $F_{1,39} = 5.42$, $P = 0.025$). The amount of algae in the guts of *Paragnetina* did not differ significantly between seasons ($P = 0.808$). The algal community in the study area was dominated primarily by cyanobacteria (*Homeothrix janthina*) and secondarily by diatoms (*Gomphonema* spp.) in the summer, and diatoms (*Melosia varians*) were most dominant in the winter. Diatoms were most frequently observed algae in the stonefly guts (*Gomphonema* spp. and *Cymbella minuta* in summer, and *Melosia varians* in winter).

The amount of animal prey in the guts of all stonefly species did not differ between seasons (two-way ANOVA: species, $F_{2,124} = 0.48$, $P = 0.623$; season, $F_{1,124} = 0.462$, $P = 0.498$; interaction, $F_{2,124} = 1.03$, $P = 0.359$; Fig. 3a). Our gut content analysis showed that there was a taxon-specific prey consumption in summer (Fig. 3b). The main prey were *Baetis* and Chironomidae for *Oyamia*, all prey taxa for *Paragnetina*, and *Baetis* for *Kamimuria*. In winter, all stonefly species mainly consumed Chironomidae. The diet composition (% contribution of prey) differed significantly between seasons for all stonefly species [one-way MANOVA: *Oyamia*, Hotelling-Lawley's (H–L) trace = 0.436, $F_{3,26} = 3.78$, $P = 0.022$; *Paragnetina*, H–L trace = 0.844, $F_{3,23} = 6.47$, $P = 0.002$; *Kamimuria*, H–L trace = 1.234, $F_{3,11} = 4.53$, $P = 0.027$]. In the diet of *Oyamia*, the percentage of *Baetis* in the summer was significantly higher than that in the winter (one-way ANOVA: $F_{1,28} = 11.61$, $P = 0.002$), whereas the percentage of *Epeorus* and Chironomidae did not differ between seasons ($P > 0.231$ for both species). In the diet of *Paragnetina*, the percentage of Chironomidae in winter was significantly higher than that in summer ($F_{1,25} = 15.45$, $P < 0.001$), whereas the percentages of *Baetis* and *Epeorus* did not differ between seasons ($P > 0.367$ for both species). In the diet of *Kamimuria*, the percentage of *Baetis* in summer was significantly higher than that in winter ($F_{1,13} = 9.03$, $P = 0.010$), the percentage of Chironomidae in winter was significantly higher ($F_{1,13} = 6.43$, $P = 0.025$), and the percentage of *Epeorus* did not differ between seasons ($P = 0.926$).

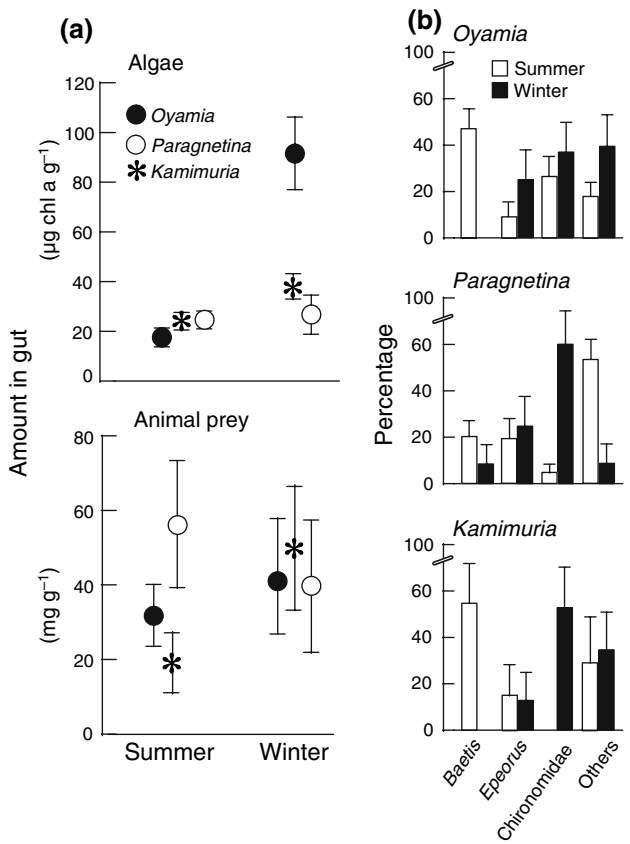

Fig. 3 **a** Amount (mean ± SE) of algae (measured as chlorophyll *a*) and animal prey (mg dry mass) in the stonefly gut per gram dry mass of a stonefly nymph. **b** Animal prey composition (mean ± SE) in stonefly guts. Others were mainly *Glossosoma* spp. (Trichoptera), *Cincticostella* spp. (Ephemeroptera) and Simuliidae (Diptera). *Open and filled bars* Summer and winter, respectively

The seasonal change in respiration rate of the stonefly nymphs differed significantly between species (two-way ANOVA: species, $F_{2,56} = 4.75$, $P = 0.013$; season, $F_{1,56} = 91.70$, $P < 0.001$; interaction, $F_{2,51} = 14.29$, $P < 0.001$; Fig. 4). *Oyamia* and *Paragnetina* had six- and twofold higher respiration rates, respectively, in the summer than in the winter (*Oyamia*, $F_{1,22} = 104.55$, $P < 0.001$; *Paragnetina*, $F_{1,18} = 43.32$, $P < 0.001$; Fig. 4). The respiration rate of *Kamimuria* did not differ between seasons ($P = 0.077$). The seasonal change in respiration rate of animal prey differed significantly between species (two-way ANOVA: prey species, $F_{2,30} = 44.03$, $P < 0.001$; season, $F_{1,30} = 12.56$, $P = 0.001$; interaction, $F_{2,30} = 4.90$, $P = 0.014$; Fig. 4). The respiration rate of *Baetis* did not differ between seasons ($P = 0.505$). *Epeorus* and Chironomidae had four- and threefold higher respiration rates, respectively, in the summer than in the winter (*Epeorus*, $F_{1,11} = 7.92$, $P = 0.017$; Chironomidae, $F_{1,11} = 25.48$, $P < 0.001$; Fig. 4).

Physiological activity is known to decline with body mass, and the mean body mass of *Oyamia* was greater in the winter (82 mg dry mass) than in the summer (30 mg). The physiological activity of larger *Oyamia* in the winter (0.4 mg O_2 g^{-1} h^{-1}) was considerably lower than that calculated with the effect of body mass on physiological activity [1.1 mg O_2 g^{-1} h^{-1} for a nymph of 82 mg dry mass, calculated assuming a slope of 0.75 in the $\log_e$(respiration rate)–$\log_e$(mg dry mass) relationship according to Peters (1983)].

Discussion

The present study used a two-source-based mixing model based on stable isotope analysis to investigate the possibility that carnivorous animals can shift seasonally to omnivory under conditions of low water temperature. This seasonal shift between carnivory and omnivory is

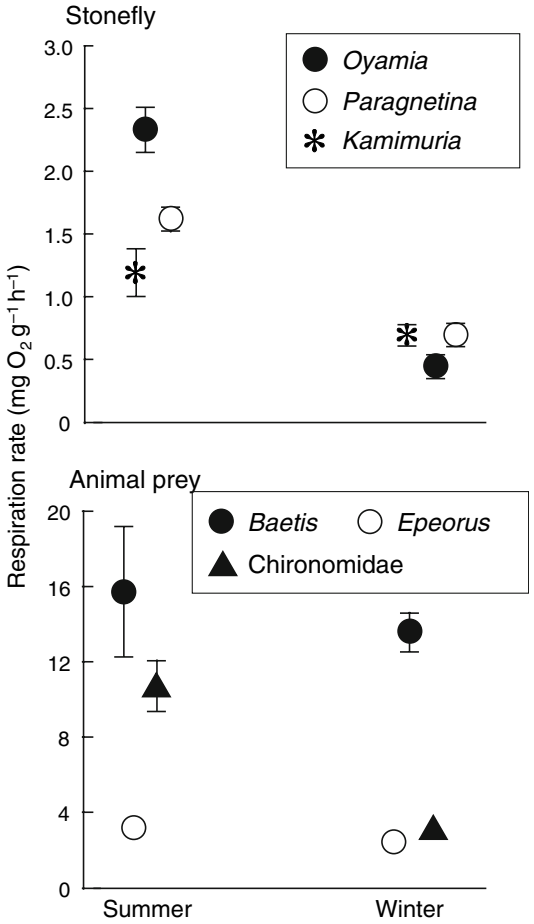

Fig. 4 Respiration rates per gram dry mass (mean ± SE) of three stonefly species and three animal prey measured during the summer and winter

not associated with an ontogenetic shift from algivory to insectivory (Feminella and Stewart 1986) because we used only large-bodied individuals at later nymphal stages. Interestingly, there were two different food-use patterns. *Oyamia* and *Paragnetina* shifted from carnivory in summer to omnivory in winter, whereas *Kamimuria* was an omnivore throughout the year. Lancaster et al. (2005) showed that some large-bodied stoneflies were omnivores based on their stable isotope analysis, but there were no obvious seasonal patterns in diet based on their gut content analysis. In our study, chlorophyll *a* was found in the gut content analysis irrespective of season, and the species composition of diatoms in gut contents coincided with that in the natural stream.

There was no difference in the prey amount in stonefly guts between seasons, although the stable isotope analysis indicated that *Oyamia* and *Paragnetina* were more carnivorous in the summer. This discrepancy may be attributed to the difference in gut evacuation rate of stoneflies between seasons (evacuation rate in the summer is approximately twofold faster than in winter; Miyasaka and Genkai-Kato, submitted). Parts of animal prey might be more likely to remain in stonefly guts in winter. *Kamimuria* was shown by stable isotope analysis to be omnivorous both in summer and winter, and the prey amount in the gut of this species showed a tendency—although not significant—to be greater in winter (Fig. 3a). The large amount of algae in *Oyamia* guts in winter (Fig. 3a) may reflect omnivory in this species, although there was no difference in prey amount in the gut of this species between seasons. As for *Paragnetina*, which the stable isotope analysis revealed to be a carnivore in summer and an omnivore in winter, there was no difference in the amount of algae or animal prey in gut between seasons. Although not statistically significant, the amount of prey in the gut had a tendency to be greater in the summer only in this species, even taking into consideration the evacuation difference between seasons (Fig. 3a). This tendency could partly explain the carnivory of *Paragnetina* in summer. However, it should be acknowledged that the algal amount in *Paragnetina* guts in the summer did not differ from that in winter. Unfortunately, we are unable to explain this discrepancy between stable isotope and gut content results (e.g., algae in *Paragnetina* guts in summer) with our data. There is a difference in the time scale between gut content and stable isotope analyses in terms of any evaluation of diet: gut content analysis provides a snapshot of information at the time when the prey organism was captured, whereas stable isotope analysis reflects a long-term average of assimilated diet. This discrepancy shown here implies that gut content and stable isotope results are not always compatible for a number of reasons, such as differences in turnover rate between compartments in the food web and selective assimilation of some parts of the food. This discrepancy between gut content and stable isotope analyses is still an open problem, and its resolution will be an important topic in future ecological studies dealing with food webs.

Stoneflies are larger than their prey, and they may have a slower turnover of elements. Consequently, stable isotope signatures are likely to change slower in stoneflies than in the prey species. Maruyama et al. (2001) reported that the half-change period of nitrogen isotope in gobies of similar body size (0.1–0.9 g wet weight) was 1.1–3.3 months. The wet weights of stoneflies in this study were 0.08–0.93 g and, therefore, the turnover rate of stoneflies would have values close to those of the gobies. As is often the case with isotope signatures of predators and prey that are not monitored over longer time spans, the sampled prey might not show a signature representative of the prey during the time period reflected in the stonefly signature.

The gut content analysis also provides important information on the use of animal prey items. All three stonefly species primarily consumed highly mobile, sprawler species [i.e., *Baetis* and *Epeorus* according to Merritt and Cummins (1996)] in summer, and their prey items shifted to less-mobile, sessile species (i.e., Chironomidae) in winter (Fig. 3b). The shift of prey items in stonefly guts corresponded well to results from measurements on physiological activity, as estimated from respiration (Fig. 4). *Kamimuria* did not show a change in

physiological activity (Fig. 4) or in stable isotope results (Fig. 1). The declines in physiological activity of *Oyamia* and *Paragnetina* during the winter were remarkable. Among animal prey, the physiological activity of *Baetis* in the winter was similar to that in summer. It is conceivable that *Baetis* was hard to capture for stoneflies with reduced physiological activity. Although our physiological activity results showed that Chironomidae also decreased their activity in winter, they were sessile species within algal mats and, therefore, were perhaps more accessible to stoneflies in the winter. Moreover, the density of Chironomidae was particularly high in winter (Fig. 2). These results imply that stoneflies foraged on a mixture of Chironomidae and algae during the winter.

The results of our study suggest that physiological activity is one of several potential causes for a diet shift. Temperature is a primary physical factor affecting animal behavior and distribution through its influence on other physical factors, such as dissolved oxygen (Genkai-Kato et al. 2000, 2005). Temperature has the potential to even alter trophic networks and ecosystem characteristics not only in aquatic ecosystems (e.g., Genkai-Kato and Carpenter 2005) but also in terrestrial ecosystems (e.g., Zeng et al. 1999). This study supports that the observation ecosystem structures may change along with changes in temperature even if the species composition of the ecosystem does not change (Kishi et al. 2005).

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Appendix 1

Table 2 Sample numbers of three stonefly species for individual dry weight, stable isotope, diet, and physiological activity analyses

Stonefly	Season	Analyses					
		DW[a]	SI[b]	Chl[c]	Prey[d]	RR[e]	Total[f]
Oyamia	Summer	59	48	20	29	12	159
	Winter	44	25	20	20	12	121
Paragnetina	Summer	36	33	20	30	12	111
	Winter	17	6	18	18	8	67
Kamimuria	Summer	20	21	20	23	12	83
	Winter	29	20	21	10	6	86

[a] Dry weight measurement
[b] Stable isotope analysis
[c] Chlorophyll *a* measurement in stonefly gut
[d] Prey items in stonefly gut
[e] Respiration rate measurement
[f] Head capsule width of all individuals was measured

Appendix 2

Table 3 Mean individual dry weight (mg) of 20 animal prey taxa in summer and winter

Order	Prey category (family or genus)	Season	Mean ± SE	*n*
Ephemeroptera	*Ameletus*	Winter	4.338 ± 0.787	10
	Baetis	Summer	0.387 ± 0.066	21
		Winter	0.222 ± 0.041	7
	Cinygmula	Summer	0.817 ± 0.116	11
		Winter	1.057 ± 0.269	6
	Epeorus	Summer	4.470 ± 0.701	19
		Winter	5.330 ± 0.762	20
	Paraleptophlebia	Summer	0.200 ± 0.036	10
	Cincticostella	Winter	5.782 ± 0.888	11
	Drunella	Summer	1.994 ± 0.278	10
		Winter	14.066 ± 0.935	10
	Ephemera	Summer	2.974 ± 1.760	2
Plecoptera	Choloroperlidae	Winter	5.219	1
	Leuctridae	Summer	1.223 ± 0.392	10
		Winter	20.551 ± 12.653	4
Trichoptera	Glossosomatidae	Summer	0.979 ± 0.193	22
		Winter	1.546 ± 0.448	15
	Goeridae	Summer	0.756	1
		Winter	1.013 ± 0.194	4
	Hydropsychidae	Summer	1.437 ± 0.376	10
		Winter	5.146 ± 0.953	6
	Rhyacophilidae	Summer	1.922 ± 0.611	13
		Winter	5.734 ± 1.965	8
Neuroptera	*Protohermes*	Summer	186.314 ± 28.776	9
		Winter	109.024 ± 30.238	5
Diptera	Blepharoceridae	Winter	15.493 ± 4.620	10
	Chironomidae	Summer	0.110 ± 0.014	11
		Winter	0.449 ± 0.111	16
	Elmidae	Winter	0.680	1
	Simuliidae	Summer	0.362 ± 0.042	10
	Tipulinae	Summer	1.651 ± 0.424	9
		Winter	17.306 ± 0.733	4

Individual prey samples were dried at 60°C for 24 h and cooled in a desiccator before weight measurement
n, Sample number

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