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Seasonal change in the gut clearance rate of three stonefly species in a Japanese stream

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Abstract We measured seasonal changes in the gut clearance rate of three stonefly species in a stream. The rate declined with water temperature. Based on the gut clearance rate obtained here, we estimated the half-reduction time of gut contents and consumption rate of stoneflies on algae. Irrespective of stonefly species, the half-reduction time in winter (2 days) was twice as long as that in summer (1 day). The areal consumption rates of stoneflies varied depending on species and season. These results imply that the seasonal consumption impact of aquatic insects can be different, despite their daily ration being similar among species.

Keywords Stonefly · Gut clearance rate · Stream · Water temperature · Consumption rate

Introduction

Food is the source of energy and material for production and activity of animals, so quantification of feeding ecology, often expressed as consumption rate, clearance rate or daily ration, is fundamental in understanding the material cycling in food webs (Båmstedt et al. 2000). In aquatic ecosystems, consumption rates of fishes have been well studied because of their commercial and recreational importance (e.g., Elliott 1972; Kawaguchi et al.

2007). There are a few studies that have measured gut clearance rates of aquatic insects (Allan 1982; Cowan and Peckarsky 1994), but seasonal changes in the rates have not been well documented.

Feeding activity of aquatic animals is strongly affected by water temperature and therefore fluctuate seasonally (Kawaguchi and Nakano 2001). Consumption rates are dependent upon the rate at which the gut contents are evacuated, and, in general, the gut contents decrease exponentially over time (Wootton 1998). Stoneflies are important foragers, influencing biomass, behavior, growth and fecundity of aquatic insects such as mayflies at lower trophic levels (Peckarsky 1980, 1996; Peckarsky et al. 1993). It is difficult to estimate exactly consumption rates of aquatic insects from animal prey components in the gut. Gut contents will underestimate both the biomass consumed as well as the variety of components, since some diet items may go unrecognized, or only soft parts may be ingested (Hershey and Peterson 1996). Stoneflies have been viewed as carnivorous, but some plant material is often found in their guts (e.g., Hynes 1970). They may be viewed as omnivorous rather than true carnivores even at later nymphal stages (Lancaster et al. 2005; Miyasaka and Genkai-Kato 2008). Chlorophyll is a good indicator of algal amount, independent of algal species composition, even in the guts (Cowan and Peckarsky 1990; Miyasaka and Genkai-Kato 2007). In this study, gut clearance rates were measured using chlorophyll rather than animal prey components to estimate the temperature-dependent seasonal consumption rates of omnivorous stoneflies. Then, we further estimated the seasonal consumption pressure of stoneflies on the lower trophic levels.

Methods

Sampling was conducted in Kuro-kawa River (35°53'N, 137°40'E) in Kiso, Nagano Prefecture, central Japan, in summer (18–25 July 2004) and winter (16–22 December

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2005) during the daytime (10:00–17:00 h). Kuro-kawa River (14 km long) is a third-order mountain stream, tributary to the Kiso River system flowing into the Pacific Ocean. Kuro-kawa means black river, named after its high algal productivity (thick, black algal mats on streambed stones) throughout the year despite the clear water. A 0.5-km stretch (6–12 m wide, 15–40 cm deep, 840 m in altitude, 2.6% gradient) of the river, 9 km downstream from the headwater, was selected as the study area for collecting organisms.

We focused on three species of stonefly nymphs (Perlidae), *Oyamia lugubris* McLachlan, *Paragnetina tinctipennis* McLachlan and *Kamimuria tibialis* Pictet, which were the dominant stoneflies in the stream. Stonefly nymphs were collected using a quadrat net (20 × 20 cm, 1.9-mm mesh) from riffle habitats at random in the study area, and all nymphs were immediately transported to the laboratory of Kiso Biological Station, Kyoto University. The nymphs for measurement of the initial chlorophyll amount in the gut (starvation time at 0 h) were immediately stocked in a freezer in the laboratory. The other nymphs were stocked in cubic cages (10 × 10 × 10 cm, 1.25-mm mesh) in Chigono-sawa Stream beside the laboratory (35°50'N, 137°41'E), so stocked nymphs were not given any food. Chigono-sawa Stream (1.5 km long, 1–2 m wide) is a first-order mountain stream, tributary to the Kiso River system through Kuro-kawa River. Nymphs for later chlorophyll measurement were grouped and stocked according to species and starvation time [24 and 48 (or 72) h in summer, and 60 and 110 h in winter] in each cage (sample numbers in each cage were listed in Table 1). At each starvation time, corresponding nymphs were transferred and stocked in a freezer for later analyses of body size and chlorophyll in the gut measurements. We measured total length (mm) to the nearest 0.1 mm with a digital caliper (Digimatic Caliper, series no. 500, Mitsutoyo, Kawasaki) using frozen specimens. Dry weight of stonefly nymphs was calculated from their total length based on Genkai-Kato and Miyasaka (2007). We conducted chlorophyll extraction from the gut of defrosted specimen. The gut of a specimen was carefully taken out of the body with forceps and placed

on a paper filter (Whatman 540). The paper filter was put into 90% acetone solution for 24 h under dark, cool conditions. The amount of algae in the gut was measured as chlorophyll (chlorophyll *a* + pheopigment) by the Unesco method (Unesco 1966) using a spectrometer (U-1100, Hitachi, Tokyo) according to the method described in Miyasaka and Genkai-Kato (2007). The amount of algae in the gut was expressed as chlorophyll per unit dry weight of a stonefly nymph.

To estimate the gut clearance rate of stoneflies at different water temperatures, we plotted the mean of chlorophyll amount in the gut per 1 g dry weight of stonefly nymph (Chl) against the starvation time (*t* h) (Fig. 1). Following Elliott (1972), the relationship between Chl and *t* was

$$\log_e \text{Chl} = \log_e \text{Chl}_{\text{ini}} - Rt,$$

where \log_e was the natural logarithm, Chl_{ini} was the initial chlorophyll amount in the gut (i.e., starvation time at 0 h), and *R* was the slope of regression line. The slopes (*R*) were compared between seasons and species using analysis of covariance (ANCOVA) with chlorophyll amount in the gut as a dependent variable, starvation time as a covariate and season or species as a main factor. If the interaction effect of season or species by starvation time was significant at *P* = 0.05, we assumed that *R* was different between season or species. All analyses were conducted using SPSS (version 11.5J).

Based on the gut clearance rates, we calculated the half-reduction time (i.e., reduction time to 50% of the initial chlorophyll amount) defined as $t_{50\%} = \log_e 2 / R$. The per capita chlorophyll amount at *t* = 0 is given by $\text{Chl}_{\text{ini}} \times \text{DW}$, where DW is the mean individual dry weight of stoneflies. The chlorophyll amount evacuated in $t_{50\%}$ (h) is $\text{Chl}_{\text{ini}} \times \text{DW} / 2$. Thus, the per capita consumption rate per day (CR_{ind} , $\mu\text{g chl ind}^{-1} \text{d}^{-1}$) is given by the following formula:

$$\text{CR}_{\text{ind}} = \frac{\text{Chl}_{\text{ini}}}{2} \text{DW} \frac{24}{t_{50\%}}.$$

Using the mean stonefly density data in Kuro-kawa River (Miyasaka and Genkai-Kato 2008), we also estimated the areal consumption rate using the formula $\text{CR}_{\text{area}} = \text{CR}_{\text{ind}} \times \text{mean density}$ (CR_{area} , $\mu\text{g chl m}^{-2} \text{d}^{-1}$).

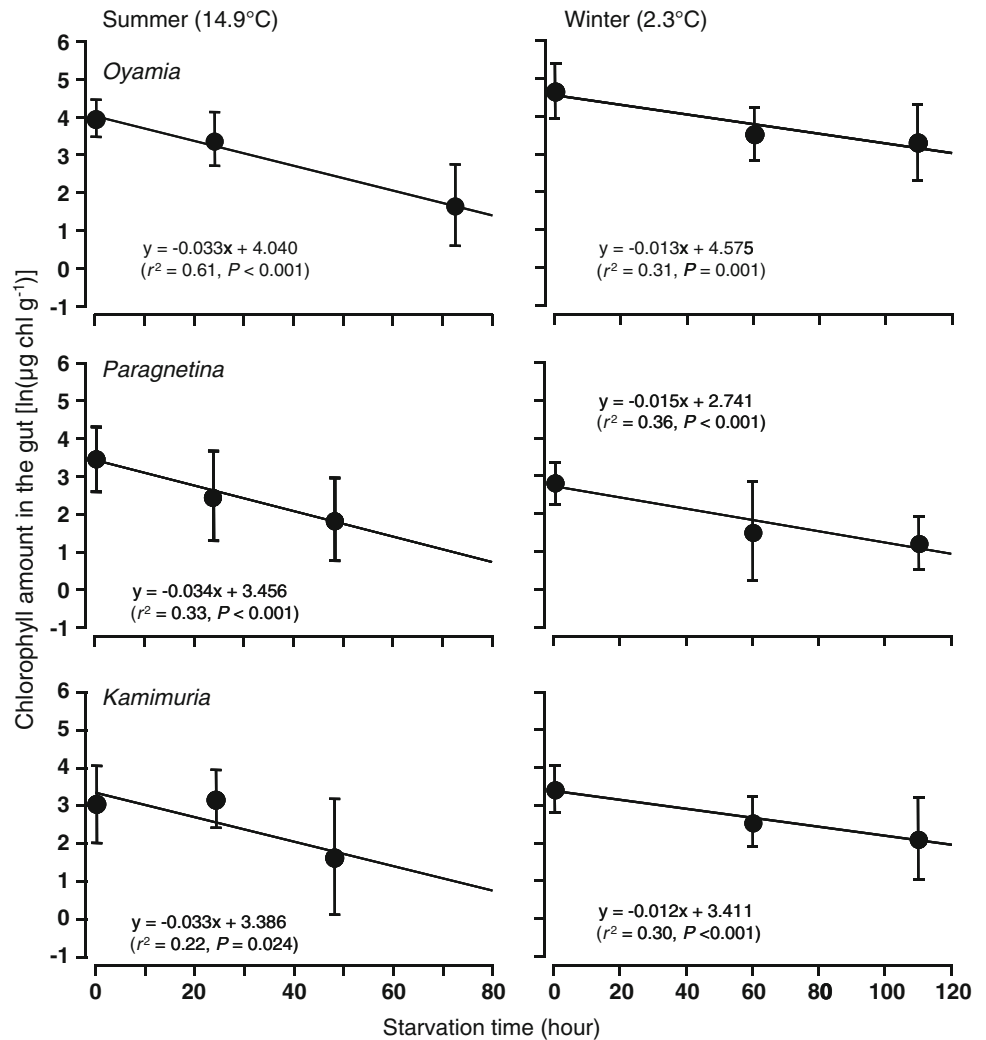
Table 1 Sample numbers (*n*) of stonefly nymphs for chlorophyll measurement grouped and stocked according to species and starvation time (*t* h) in each cage

Species	Summer		Winter	
	<i>t</i>	<i>n</i>	<i>T</i>	<i>n</i>
<i>Oyamia</i>	0	13	0	14
	24	14	60	15
	72	17	110	13
<i>Paragnetina</i>	0	10	0	13
	24	8	60	12
	48	13	110	15
<i>Kamimuria</i>	0	6	0	11
	24	6	60	11
	48	11	110	14

Results and discussion

There was no significant difference in water temperature between Kuro-kawa River (mean ± SD, 15.1 ± 0.7°C in summer, *n* = 7; 2.3 ± 0.6°C in winter, *n* = 9) and Chigono-sawa Stream (14.9 ± 0.7°C in summer, *n* = 7; 2.3 ± 0.9°C in winter, *n* = 5) in summer (*P* = 0.583) or in winter (*P* = 0.995). The chlorophyll amount in the gut declined significantly with time for all species in both seasons (Fig. 1). Two-way ANCOVA revealed that *R* differed significantly between seasons, with interaction effect of season × starvation time being significant

Fig. 1 Relationship between starvation time (t h) and the log-transformed values of chlorophyll amount (Chl, $\mu\text{g chl per 1 g of dry weight of stonefly nymph, mean} \pm \text{SD}$) in the gut. Lines were fitted by a simple linear regression



(Table 2). For *Oyamia* and *Paragnetina*, R in summer was significantly steeper than that in winter by a significant interaction effect of season by starvation time ($F_{1, 82} = 13.99$, $P < 0.001$ for *Oyamia*; $F_{1, 67} = 5.02$, $P = 0.028$ for *Paragnetina*; Fig. 1), whereas there was no significant interaction effect for *Kamimuria* ($P = 0.158$). The significant interaction effect indicates that R differed significantly between seasons. The differences in gut clearance rate between seasons for *Oyamia* and *Paragnetina* may be related to the tendency of their food resource to shift from an omnivorous diet (algal foods + animal foods) in winter to a more carnivorous diet (animal foods > algal foods) in summer (Miyasaka and Genkai-Kato 2008). The effect of animal materials, which was not addressed by this study, may alter the gut clearance rate of the stoneflies. There was no significant interaction effect of species by starvation time on R in summer ($P = 0.993$) or in winter ($P = 0.807$), indicating that R did not differ between stonefly species in each season.

The half-reduction time ($t_{50\%}$) was 21 h for *Oyamia*, 20 h for *Paragnetina*, and 21 h for *Kamimuria* in

Table 2 Results of ANCOVA for the effects of season, species, and starvation time (covariate) on the chlorophyll amount in the stonefly gut

Source	df	F	P
Season	1	0	0.995
Species	2	16.911	< 0.001
Time	1	96.497	< 0.001
Season \times species	2	4.186	0.017
Species \times time	2	0.112	0.894
Season \times time	1	14.299	< 0.001
Season \times species \times time	2	0.48	0.953
Error	204		

summer; 53 h for *Oyamia*, 46 h for *Paragnetina*, and 58 h for *Kamimuria* in winter. There was a tendency, regardless of stonefly species, that the reduction time to the half amount in winter (≈ 2 days) was twice as long as that in summer (≈ 1 day). The $t_{50\%}$ values of stoneflies were comparable to those of fishes measured under natural conditions: 14 h at 12°C and 115 h at 2°C for rainbow trout (Kawaguchi et al. 2007), and 4 h at 12°C and 19 h at 2°C for sculpin (Miyasaka et al. 2005). The

Table 3 Dry weight, density, and consumption rates of three stonefly species used in the experiments in each season

Season	Species	Dry weight ^a (mg)	Density ^b (m ⁻²)	CR _{ind} ^c	CR _{area} ^d
Summer	<i>Oyamia</i>	20.8 ± 0.8 (44)	4.0 ± 1.0	0.69	2.76
	<i>Paragnetina</i>	68.9 ± 5.7 (31)	1.3 ± 0.5	0.71	0.93
	<i>Kamimuria</i>	26.7 ± 2.2 (23)	3.0 ± 0.8	0.55	1.65
Winter	<i>Oyamia</i>	65.4 ± 5.9 (42)	18.5 ± 2.8	2.34	43.36
	<i>Paragnetina</i>	36.9 ± 2.6 (42)	1.0 ± 0.5	0.42	0.42
	<i>Kamimuria</i>	18.7 ± 1.0 (36)	3.6 ± 1.1	0.14	0.52

Values are mean ± SE (sample number)

^aData calculated from body length based on Genkai-Kato and Miyasaka (2007)

^bData from Miyasaka and Genkai-Kato (2008) measured in the same study area of Kuro-kawa River ($n = 245$ in summer, $n = 135$ in winter)

^cPer capita consumption rate (μg chl ind⁻¹ d⁻¹)

^dAreal consumption rate (μg chl m⁻² d⁻¹)

difference in $t_{50\%}$ between summer and winter was smaller for stoneflies (≈ 2 times) than fishes (> 4 times).

Although all three species took similar R and $t_{50\%}$ values in each season, their per capita and areal consumption rates varied considerably depending on species and season (Table 3). Estimates of areal consumption rates by animals are essential for studies modeling quantitatively the energy flow at the basal trophic level in ecosystems. The changes in spatial distribution and dynamics of algal communities are determined by bottom-up activities (areal primary production; e.g., Nakanishi and Yamamura 1984) and top-down forces (areal grazing rate; e.g., Abe et al. 2007). The variation in consumption rate is attributed to their seasonal characteristics of body size and dominance in abundance. Specifically, *Oyamia lugubris* in winter consisted of remarkably large-bodied individuals and was dominant in density (Genkai-Kato and Miyasaka 2008). This study showed that consumption impact of animals within the same guild can be different depending on ecological characteristics such as body size and abundance, despite their physiological characteristics (i.e., gut clearance rate) not varying between them.

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