Utilization of leachate from *Quercus glauca* leaf litter and effects of feeding and case-building behaviors of *Anisocentropus* larvae on stream water composition

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The utilization of leachates from *Quercus glauca* leaf litter by larvae of shredder species, *Anisocentropus*, as well as the effects of feeding and case-building behaviors by these shredders on dissolved matter composition in streams were evaluated. To obtain highly concentrated leachate from leaf litter, 500 *Quercus glauca* leaves, 100 *Meliosma rigida* leaves and 100 *Styrax japonica* leaves were submerged in a chamber filled with 10 l of distilled water for 30 days. The 500 *Quercus glauca* leaves used for the above treatment were air-dried for 1 week and then oven-dried at 80°C for 12 h. Three chambers were prepared and 6 randomly selected leaves were weighed and placed into each of the chambers. Then, three *Anisocentropus* larvae were randomly selected in each of three groups (small size, medium size and large size group). Four sets of chambers were prepared, each with five replicates (sub-samples). Each chamber was filled with 500 ml of stream water, distilled water, leachate solution, and diluted leachate diluted to half concentration. After 30 days of incubation, weight loss rates of *Quercus glauca* leaves by decomposition, growth rates of larvae and the water physicochemical parameters in each chamber were measured.

Leaf weight loss ranged from 16 to 27% and was maximum in distilled water and least in leachate and/or diluted leachate ($P < 0.0001$). Markedly lower growth rate in distilled water indicated an extremely high leaf processing rate compared to that for other solutions ($P < 0.0001$). The results of the leaching experiment in which a leaf was divided into several pieces showed that the concentrations of K^+, Mg^{2+} and Ca^{2+} in water tended to increase with increasing number of pieces ($P < 0.05$) .

From these experimental results, it was indicated that *Anisocentropus* larvae, which have been regarded as consumers of CPOM and transformers CPOM to FPOM, UPOM and DOM in the classification of functional feeding groups among stream invertebrates, also utilize DOM leached from *Quercus glauca*, *Meliosma rigida* and *Styrax japonica* leaves, which is a relatively high quality food for survivorship and that biological fragmentation of leaves resulting from feeding and case-building behaviors of invertebrates accelerates the leaching processes of water-soluble substances, such as K^+, Mg^{2+} and Ca^{2+}.

Kye words: Stream water composition, leaf processing, shredder, leaching, *Quercus glauca* leaves.

1. INTRODUCTION

Leaf litter enters streams and becomes the primary source of energy for secondary consumers in the streams. This allochthonous organic matter is decomposed through a combination of physical, chemical and biological processes[1)], releasing inorganic nutrients[4,22)] and dissolved organic matter (DOM)[21)] into the water. Since considerable quantities of allochthonous material enter lotic ecosystems from riparian forests[9,10)], substances derived from the decomposition of leaf litter can be expected to have a major impact on stream water quality.

Soon after entering the water, leaf litter undergoes a decomposition process that can be divided into leaching, conditioning, and fragmentation. Soluble chemicals within the leaf are leached from the plant tissue, and the remaining coarse particulate organic matter (CPOM) is then colonized by fungi (primarily aquatic phyphomycetes) and bacteria[8)] in the process called conditioning. Colonized leaves are described as being conditioned, and the microbial substances that become attached to the leaf surfaces make the leaves more digestible for aquatic invertebrates[12)], thus facilitating the fragmentation process. Allochthonous organic material, such as leaf litter, provides 50 to 90% of the energy utilized by stream organisms[11)].

Among the functional feeding groups in stream invertebrates[8)], large particle detritivores act as shredders and feed predominantly on leaf litter that enters the stream food web by falling into or being blown into the stream. The feeding activity of leaf-shredding insects increases the availability of CPOM from leaf litter for more specific feeding on small particles by collectors as fine particulate organic matter (FPOM), ultrafine particulate organic matter (UPOM) and DOM. Shredders accelerate the biological conversion and fragmentation of leaf litter to FPOM and UPOM[1,29]. In addition to utilization as a source of food, leaf litter is also utilized by aquatic invertebrates as case materials for case-building, which increases opportunities for fragmentation. Great quantities of dissolved organic matter, such as ions, DOC and nitrogen are also generated through this process[18,19], but the potential importance of shredders in generating dissolved organic matter in streams and the subsequent influence of leaf fragmentation on stream water composition have not been fully investigated.

Our previous experimental results[20], however, have indicated the possibility that pre-terminal and/or terminal larval instars of *Lepidostoma japonicum*, which is classified as a shredder, utilizes leachates (DOM) from *Quercus glauca*, as well as CPOM, to support growth in stream food webs and that leachates derived from the shredding activities of insect larvae, in addition to feeding and case-building activities, affect the physicochemical characteristics of stream water.

In this study, we evaluated the utilization of leachates from *Quercus glauca* leaf litter by larvae of shredder species, *Anisocentropus* (Trichoptera), as well as the effects of feeding and case-building behaviors by these shredders on dissolved matter composition in streams.

2. Methods

2-1. Site description

Sampling for experiments conducted in the laboratory was conducted in the Takeo River in Saito City, Miyazaki Prefecture. The geomorphological and vegetational characteristics of this reach of the river were characterized in our previous study[20]. The Takeo River, a tributary of the Hitotsuse River, flows across elevations ranging from 600 to 100 m, originates in the southern Kyushu Mountains (Fig. 1). This first-order stream has a mean width of 10 m, a mean depth (at modal flow) of 0.3 m, a mean slope of 0.25, and mean current velocities in the range of 20 to 30 cm/sec. The stream bottom is primarily composed of gravel, pebbles and cobble substrates and consists of a series of riffles, pools, and side-pools with irregular meanders. The riparian vegetation consists of evergreen trees, such as *Quercus glauca*, *Symplocos theophrastifolia* Sieb. et Zucc., *Machilus japonica* Sieb. et Zucc., and *Litsea acuminata* Kurata. Since the slope of the stream basin is steep, tree leaves supplied from the forest floor tend to be transported to and collect in the stream.

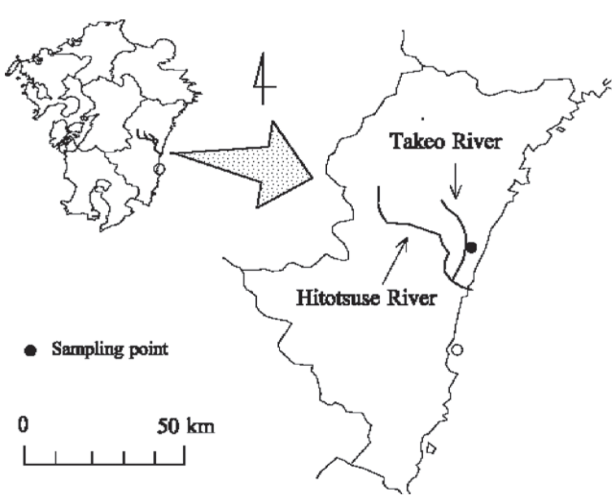

Fig. 1. Investigated site.

The invertebrate fauna of the Takeo River is rich, including over 50 taxa that have been recorded to date. During the winter and spring, *Anisocentropus* larvae are one of the most abundant aquatic invertebrates in the Takeo River[16]. Larvae are most commonly found among deposits of allochthonous organic material in lentic areas such as pools and side-pools in the stream.

2-2. Materials

Leaves from the dominant plant species and larvae from the dominant leaf-shredding macroinvertebrate species were collected from along the reach of river in the study area.

Leaves were collected from trees growing in the riparian zone of an exposed, unfertilized section of river flowing over an alluvial plain. All leaves were sun old-leaves and were picked just before abscission from old branches located at 2 to 4 m above the ground at the study site on March 1, 2014. Leaves were collected from the dominant species in the riparian zone as follows: 500 *Quercus glauca* leaves, 100 *Meliosma rigida* leaves and 100 *Styrax japonica* leaves.

Although the precise life history of *Anisocentropus* has not been described, larvae typically grow to at least 15 mm before emerging from leaf-disc cases made from a pair of ellipsoid leaf materials[15]. *Anisocentropus* belongs to the family Calamoceratidae, which includes shredder detritivores that feed upon large particulate leaf material and use leaves for case-building and habitat[24]. *Anisocentropus* larvae were most common in the spring in side-pools in the study site. Larvae were typically found associated with allochthonous organic material that had been deposited on course to fine sand in slow-flowing water. In this study, *Anisocentropus* larvae were divided into three groups according to approximate case length; small sizes had a case length of$<$15 mm, medium sizes had a case length of 16 to 25 mm, and large size had a case length of $>$26 mm. In each size group, 200 larvae were collected on March 26, 2014. Specimens were maintained in aerated stream water at ambient temperature (20°C) for 5 days to acclimate the larvae to laboratory conditions.

Stream water (100 L) was collected from the same area of the stream for the leaching experiments.

2-3. Laboratory experiments

2-3-1. Preparation of leachate from leaf litter

Leaves collected from trees were washed with distilled water to remove surface dust and atmospheric gases, air-dried for 1 week and then oven-dried at 80°C for 12 h. To obtain highly concentrated leachate from leaf litter[17], the leaves were submerged in a chamber filled with 10 l of distilled water for 30 days. Solution obtained in this process was used as leachate in other experiments. As the cuticle of *Quercus glauca* leaves is a physical obstacle to invertebrate feeding, the leaves were incubated for 30 days to degrade the cuticle and to promote conditioning and leaching[18].

2-3-2. Weight loss of *Quercus glauca* leaves and growth of *Anisocentropus* larvae in 30-day incubation experiments

The 500 *Quercus glauca* leaves used for the above treatment were air-dried for 1 week and then oven-dried at 80°C for 12h. Three chambers (25cm × 15cm × 10cm) were prepared and 6 randomly selected leaves were weighed and placed into each of the chambers. The whole leaves retained their shape without any skeletonization.

Then, three *Anisocentropus* larvae were randomly selected in each of three groups described previously (small size, medium size and large size group) and the wet weight and wet length of each *Anisocentropus* larvae case were measured after blotting water from the surface of the cases onto a napkin, and each was placed in a chamber.

Four sets of chambers were prepared, each with five replicates (sub-samples). Each chamber was filled with 500 ml of stream water, distilled water, leachate solution (leachate1), and diluted leachate solution which was diluted with distilled water to half concentration (leachate2). The water in each chamber had a water depth of approximately 2 cm, and the water in each chamber was aerated to simulate the physical environment of leaf litter in the side-pools of the stream. The larvae used in the experiments were checked daily, and the experiment was to be terminated if the animals appeared to be depleting their food supply or started to emerge.

After 30 days of incubation, all of the remaining materials and larvae were removed from each chamber. The processed leaves and leaf fragments larger than 1 mm in diameter were selected as CPOM, and leaf fragments and particles smaller than 1 mm in diameter or fine particulate organic matter were selected as FPOM using a sieve. The CPOM and FPOM fractions from each chamber were air-dried for 1 week, oven-dried at 80°C for 12 h, and weighed. The wet length and wet weight of the larval cases were measured after blotting water from the surface.

The concentration of total carbon (TC), including total organic carbon (TOC) and inorganic carbon (IC), and total nitrogen (TN) was measured with a TOC-TN analyzer (TNC-6000, Toray Engineering K.K., Japan). Cations (Li^+, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) in the water were measured by ion chromatography (DX-120, Nippon Dionex K.K., Japan).

2-3-3. Evaluation of leaching rates relative to physical fragmentation of leaves

In order to clarify whether physical fragmentation of *Quercus glauca* leaves by feeding and case-building behaviors of *Anisocentropus* larvae promotes leaching of dissolved substances from the leaves, the following experiments were conducted. Three *Quercus glauca* leaves were selected from the leaves prepared in Section 2-3-2. Each leaf had a dry weight of almost 1 g. Each leaf was not divided, divided into four equal pieces, and divided into eight equal pieces, respectively, and was placed individually into chambers filled with 1000 ml of distilled water and aerated. Each series had five replicates (sub-samples).

After 30 days of incubation, all pieces were removed from the chambers, air-dried for 1 week, oven-dried at 80°C for 12 h, and weighed at each chamber. The total carbon (TC), total nitrogen (TN) and cations (Li^+, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) in each chamber were determined by the same methods as described above.

2-3-4. Measurement of initial carbon and nitrogen content in leaves

One leaf from the group showing the maximum weight loss due to grazing and one from the minimum weight loss group were selected from each chamber. Samples were taken from each leaf in an area 5 mm to the right of the midrib in the center of the leaf. Each fragment was analyzed using an ash-free CHNS/O Analyzer (2400 Series II, Perkin Elmer, Inc., U.S.A.) to measure the carbon and nitrogen leaf content after submergence.

2-3-5. Statistical Analysis

The effects of treatment solution (stream water, distilled water, leachate (leachate1) and diluted leachate (leachate2)) and larvae size were analyzed by two-way analysis of variance. In this analysis, treatment solution and size group of *Anisocentropus* larvae in each chamber were regarded as independent variables and the measured values of weight loss rate of leaves and growth rate of cases of larvae were considered as the dependent variables. Furthermore, the differences of the concentrations of TC, TN and cations among larvae size in each treatment solution were analyzed by one-way analysis of variance.

3. Results

3-1. Leaf weight loss after incubation with larvae

Weight loss of leaves due to leaching and processing by larvae was calculated by subtracting the final weight of each leaf after incubation from the initial weight in each chamber in four types of solutions and in the presence of larvae of three size classes (Table 1). In natural stream ecosystems, FPOM is produced from the egestion of particles and mechanical fragmentation of the leaves during grazing by invertebrates. In the present experiment, however, the small number of *Anisocentropus* larvae that was placed in each chamber made only a small contribution to FPOM through the production of feces. Therefore, the data in Table 1 indicate CPOM.

Leaf weight loss and percent weight loss were maximum in distilled water and least in leachate2. Differences in weight loss for *Quercus glauca* leaves were small among insect sizes for each solution type. Two-way ANOVA of solution type and larvae size shows that significant differences were observed for solution type for both weight loss and percent weight loss ($P < 0.0001$), but size of the larvae had no effect on the weight loss of the leaves, while significant interactions were observed (Table 2).

3-2. Growth, pupation and emergence rate of *Anisocentropus* larvae

Anisocentropus larvae used *Quercus glauca* leaves for food, leaf-disc case-building, and shelter in the experiments. After 20 days, some of the larvae in the experimental chambers attached themselves onto stationary leaves or sank to the bottom of the chambers before emerging. All larvae survived the experimental period, and no dead larvae were observed.

The growth rate of *Anisocentropus* larvae during the experimental period, which was determined as the increases in the mean wet weight of individual larvae, is shown in Table 3. The wet weight of insects which pupated or emerged was excluded from the data shown in Table 3.

The growth in weight (descrived as “growth weight” in this paper) of larvae cases after the experiment ranged

Table 1. Weight loss and percent weight loss of *Quercus glauca* leaves during 30-day incubation by solution and larvae size

mean ± standard error

solution	larvae size	weight loss (g)	mean (g)	percent weight loss (%)	mean (%)
stream water	large	0.078 ± 0.006	0.066 ± 0.004	23.70 ± 2.07	20.76 ± 1.23
	middle	0.055 ± 0.005		19.31 ± 2.48	
	small	0.063 ± 0.004		19.27 ± 1.49	
distilled water	large	0.067 ± 0.007	0.076 ± 0.009	21.37 ± 3.03	27.20 ± 2.70
	middle	0.125 ± 0.024		35.06 ± 5.39	
	small	0.066 ± 0.009		25.18 ± 3.66	
leachate1	large	0.045 ± 0.002	0.053 ± 0.006	14.21 ± 1.14	17.33 ± 1.99
	middle	0.037 ± 0.003		13.59 ± 1.13	
	small	0.083 ± 0.010		24.18 ± 4.64	
leachate2	large	0.057 ± 0.008	0.046 ± 0.004	17.29 ± 1.66	16.23 ± 1.03
	middle	0.047 ± 0.004		15.63 ± 1.64	
	small	0.046 ± 0.008		15.77 ± 2.28	

Table 2. ANOVA of all physicochemical parameters for leaf weight loss and percentage leaf weight loss among treatment groups

	< leaf weight loss >				< percentage leaf weight loss >			
	n	mean square	F	*P*	n	mean square	F	*P*
solutions	3	0.003	2.798	< 0.0001	3	366.277	2.798	< 0.0001
size of larvae	2	0.001	3.191	n.s.	2	23.172	3.191	n.s.
interaction	6	0.001	2.295	< 0.001	6	146.619	2.295	< 0.01
residual error	48	0.001			48	41.243		

Table 3. Weight of larvae case and growth rates of *Anisocentropus* larvae for 30 day incubations by solution and larvae size. Case weight is evaluated as per one larva and growth rate is defined as ((final weight)／(initial weight))×100 in this paper

solutions	larvae size	weight of larvae case		growth in weight	growth rate	mean growth rate
		initial	final			
		① (g)	② (g)	②－① (g)	（②／①） × 100 (%)	(%)
stream water	large	0.14	0.26	0.12	89.1	179.6
	middle	0.09	0.23	0.14	145.9	
	small	0.06	0.22	0.16	303.7	
distilled water	large	0.13	0.12	−0.01	−7.5	9.0
	middle	0.08	0.07	−0.02	−20.9	
	small	0.05	0.07	0.02	55.3	
leachate1	large	0.14	0.19	0.05	37.6	134.5
	middle	0.10	0.18	0.09	88.8	
	small	0.04	0.16	0.12	276.9	
leachate2	large	0.16	0.21	0.05	29.6	25.7
	middle	0.12	0.14	0.02	22.8	
	small	0.06	0.08	0.01	24.8	

Table 4. Pupation and emergent rates of *Anisocentropus* larvae for 30 day incubation by size and solution

（%）

solution	larvae size	pupation rate ①	emergence rate ②	total ①+②	mean
stream water	large	13.3	33.3	46.7	35.6
	middle	13.3	33.3	46.7	
	small	0.0	13.3	13.3	
distilled water	large	20.0	66.7	86.7	55.6
	middle	13.3	33.3	46.7	
	small	13.3	20.0	33.3	
leachate1	large	20.0	46.7	66.7	51.6
	middle	20.0	33.3	53.3	
	small	0.0	33.3	33.3	
leachate2	large	33.3	33.3	66.7	40.0
	middle	20.0	33.3	53.3	
	small	0.0	0.0	0.0	

Table 5. ANOVA of growth in weights and growth rates of cases by treatment solution and size of larvae

	＜ growth in weight of case ＞				＜ growth rate of case ＞			
	n	mean square	F	*P*	n	mean square	F	*P*
solutions	3	0.060	2.798	＜ 0.0001	3	103367.9	2.798	＜ 0.0001
larvae size	2	0.004	3.191	n.s.	2	93684.6	3.191	＜ 0.0001
interaction	6	0.002	2.295	n.s	6	3.7	2.295	＜ 0.01
residual error	48	0.001			48	41.243		

from 0.12 to 0.16 g, −0.02 to 0.02 g, 0.05 to 0.12 g, and 0.01 to 0.05g, in stream water, distilled water, leachate1 and leachate2, respectively. In each treatment, small larvae have relatively faster growth, except for chambers with leachate2. The ranking of mean growth rate by solution type was as follows: stream water＞leachate 1＞leachate 2＞distilled water. The markedly lower growth rate in distilled water indicated an extremely high leaf processing rate compared to that for other solutions.

Given the possibility of some leaf cases being ingested by either the individual or another *Anisocentropus* after the building new cases based on the negative growth weight of larvae of large and medium sizes in some chambers, especially in the distilled water chambers, considerable damage to cases was expected in treatments with relatively scarce food resources. However, no naked larvae were observed in any of chambers.

Pupation and emergence rates of larvae over the course of the experiment by larvae size and incubation solution type are summarized in Table 4. The mean summed pupation and emergence rate was lowest in stream water, and no small larvae pupated and emerged in chambers with leachate2 solution type. Both pupation rates and emergence rates tend to increase with increasing larvae size. The mean summed pupation and emergence rate was highest for distilled water, indicating that the larvae were inclined to emerge rapidly. However, none of the differences among treatments in mean pupation and emergence rates were statistically significant.

Table 5 shows the results of two-way ANOVA for growth weight and growth rates of cases. In this analysis, solution (stream water, distilled water, leachate1 and leachate2) and larvae size were treated as independent variables, and larvae growth weight and growth rate of cases were considered as the dependent variables. Significant differences among treatments and larvae size were observed in growth rates of cases ($P<0.0001$) with significant interaction ($P<0.01$) .

3-3. Carbon and nitrogen content of leaves after 30 days of incubation

The carbon and nitrogen content of the *Quercus glauca* leaves of which percent weight loss were maximum and minimum in each chamber corresponding to each treatment are shown in Table 6. A relatively wide variety of compounds were observed in carbon content of stream water. The mean carbon content in the stream water, distilled water, leachate1 and leachate2 was 47.6%, 42.2%, 46.0%, and 41.4%, respectively. The carbon of *Quercus glauca* leaves measured just before submergence in our previous study[19] had a similar carbon content (46%), indicating that relatively soluble carbon was leached from the leaves for submerged period in the present experiment. The mean nitrogen content was 2.4%, 2.2%, 2.5%

Table 6. Ranges and mean values (%) for carbon and nitrogen content for leaves selected from each treatment

（%）

	carbon contents		nitrogen contents	
	range	mean	range	mean
stream water	33.9 － 62.7	47.6	1.6 － 3.2	2.4
distilled water	25.9 － 45.7	42.2	1.5 － 2.6	2.2
leachate1	42.0 － 47.7	46.0	2.2 － 2.9	2.5
leachate2	35.0 － 47.5	41.4	1.8 － 2.5	2.2

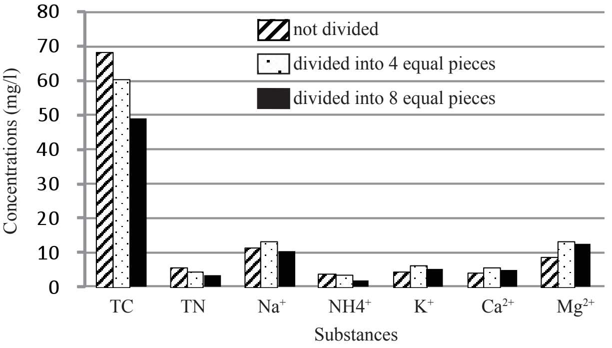

Fig. 2. Comparison of concentration of substances leached from leaves divided into the indicated number of pieces.

and 2.2%, respectively compared to 2.0% in our previous study[19]. The higher accumulation of nitrogen in this experiment during the incubation may have accelerated leaf decomposition in the experiment.

3-4. Change in concentrations of total carbon, total nitrogen and cations in each solution accompanied with leaf decomposition

Initial (before submergence of *Quercus glauca* leaves) and final (after 30 days of incubation) concentrations of TC (total carbon), TN (total nitrogen) and cations in each chamber corresponding to solutions and larvae size are shown in Table 7. In the present study, because no IC was detected in any of the samples, TC concentration was equal to TOC concentration.

In each treatment, components with significant differences ($P < 0.05$) among larvae size were Na^+ and K^+ in stream water, TC, TN, Na^+ and K^+ in leachate1, and TC in leachate2. In distilled water, significant differences ($P < 0.05$) were observed in all substances except for TN. No Li^+ was detected in the experiments.

3-5. Change of dissolved-substances leaching-rates from divided *Quercus glauca* leaves

Figure 2 shows the concentrations of TC, TN and cations leached from *Quercus glauca* leaves in each chamber after 30 days of incubation. The concentrations of TC, TN and NH_4^+ in water were higher than in submerged leaves that were kept intact or divided into four equal pieces. On the other hand, the concentrations of K^+, Mg^{2+} and Ca^{2+} in water tended to increase when leaves were divided. Significant correlations ($P < 0.05$) were observed among treatments in all substances.

Table 7. Initial and final concentrations (mg/l) of TC, TN and cations for each incubation treatment. Final concentrations are reported as the mean ± standard deviation.

（mg/l）

		TC	TN	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
＜ initial ＞								
stream water		6.23	0.87	4.65	0.84	0.76	1.98	5.90
distilled water		0.00	0.00	0.00	0.00	0.00	0.00	0.00
leachate1		9.40	0.72	1.65	0.93	2.09	0.60	1.76
leachate2		4.70	0.36	0.82	0.46	1.05	0.30	0.88
＜ final ; after 30 days of incubation ＞								
stream	large	16.43±4.40	0.92±0.27	1.52±0.44	0.31±0.03	2.11±0.09	0.46±0.05	0.60±0.05
	middle	12.65±2.01	0.67±0.13	1.27±0.14	0.44±0.05	3.74±0.50	0.51±0.10	0.53±0.07
	small	11.52±1.73	0.79±0.14	2.28±0.13	0.21±0.03	4.13±0.77	0.43±0.06	0.59±0.23
distilled	large	8.30±0.70	0.46±0.03	0.29±0.03	0.43±0.04	2.69±0.05	0.14±0.02	0.82±0.01
	middle1	3.61±0.90	0.55±0.06	0.29±0.02	0.55±0.05	2.29±0.08	0.11±0.01	0.17±0.01
	small	9.78±0.84	0.54±0.02	1.78±0.59	0.36±0.02	1.92±0.26	0.14±0.01	0.15±0.01
leachate1	large	11.56±0.37	0.54±0.01	0.65±0.17	0.30±0.04	2.63±0.07	0.11±0.01	0.16±0.02
	middle	11.33±1.09	0.52±0.03	0.38±0.03	0.31±0.02	2.11±0.32	0.13±0.01	0.18±0.02
	small	15.36±0.12	0.80±0.02	1.61±0.25	0.40±0.02	2.90±0.09	0.11±0.01	0.21±0.01
leachate2	large	7.32±0.26	0.44±0.04	1.25±0.35	0.32±0.04	2.48±0.08	0.22±0.03	0.34±0.04
	middle	8.97±0.18	0.54±0.01	0.64±0.03	0.38±0.02	2.43±0.02	0.20±0.01	0.28±0.01
	small	9.57±0.96	0.55±0.07	0.58±0.03	0.40±0.05	2.42±0.10	0.23±0.02	0.29±0.03

4. Discussion

4-1. Overall description of the previous experiment results used *Quercus glauca* leaves and *Lepidostoma japonicum* larvae[20]

Quercus glauca leaves collected at the same study site on March 19, 2013 were well washed and dried. 1, 3, 6, 12, and 24 randomly selected leaves were then weighed and placed into each of the five chambers filled with 1000 ml of stream water, respectively. Control and experimental treatments were prepared. After conditioning, six similar-sized larvae pre-terminal or terminal larval instars were placed in each of the experimental chambers. After 30 days of incubation, weight loss rates of leaves by decomposition and the water physicochemical parameters were measured. Weight loss in leaves by leaching was 14.95－27.46% and the overall net percentage processing by the larvae was approximately 23－50% according to the intial weight of leaves. Physicochemical parameters were observed to increase in leachate in stream water samples and significant differences between control and experimental treatments were observed in percentage processing of leaves, EC, pH, TC, TN, Na^+, NH_4^+, K^+, Cl^- and NO_3^- ($P<0.05$). It is mainly due to leaching of water-soluble organic substances from leaves that were fragmented due to shredding and case-building by the larvae and increased microbial populations and fungi generated from feces of the larvae. Furthermore, higher larval growth and emergence rates, and lower larval mortalities, were observed in chambers containing more leaves and in which leachate concentrations were relatively higher. From these experimental results, the possibility that leachates from the *Quercus glauca* leaves are utilized by pre-terminal and/or terminal larval instars of *Lepidostoma japonicum* for growth and that leachates derived from the shredding activities of insect larvae affect the physicochemical characteristics of stream water were demonstrated.

4-2. Weight loss of *Quercus glauca* leaves

In deciduous leaves, weight loss due to leaching occurs within a few days of submergence. For example, autumn shade leaves submerged in water can lose up to 40% dry weight in a few days[14] . Similarly, sugar maple, yellow birch, and beech showed an ash-free dry weight of 85.4% of the initial weight after 2 days of submergence in the laboratory[24]. Webster *et al*.[30] reported that up to 25% of the initial dry weight of some riparian deciduous tree leaves (e.g., *Alnus* sp., *Salix* sp.) was lost by leaching within the first 24 h of submergence. Contrary to those weight loss rates of autumn-shade leaves, weight loss in evergreen leaves from *Quercus glauca* after 30 days of submergence in our previous study was 14－27%[18,20]. However, all of these data were collected in invertebrate-free experiments.

In this study, weight loss from leaves was broadly defined as the result of both leaching and larval-induced leaf weight loss. It is difficult to precisely distinguish the percent weight loss due to grazing and case building. In the experimental chambers in this study, the percent weight loss of leaves ranged from 16% to 27%. Approximately 8% to 10% of the weight loss is considered to be due to microbial processing[13], but water in each chamber in this experiment was almost microbial-free, except for the chambers with stream water. It is therefore reasonable to assume that the weight loss of *Quercus glauca* leaf litter is attributed to leaching and consumption by *Anisocentropus* larvae for feeding and case building.

The ranges of weight loss rates of leaves in the presence of *Anisocentropus* larvae in this experiment were not markedly different from those in invertebrates-free experiments. This may be due to, in part, to insufficient conditioning of the leaves used in this experiment. At least two mechanisms appear to be responsible for the conditioning effect: microbial production and microbial catalysis[12] and colonization by fungi may strongly influence palatability of leaves and affect subsequent feeding rates of inveretebrates. Several stream-dwelling detritivores appear to prefer conditioned rather than freshly fallen or sterile leaves[12]. It is, therefore, possible that the conditioning of *Quercus glauca* leaves used in this experiment was worse than that used in previous experiments. The cuticle of *Quercus glauca* leaves may be a physical obstacle to invertebrate feeding, and it gradually disappears as a result of conditioning. The 30-day incubation of leaves for leachate preparation was insufficient to achieve complete leaching of cuticle from *Quercus glauca* leaves used in this experiment[17,18].

This hypothesis is supported by the observed C/N ratio of the selected leaves. Rapid decomposition may be associated with the rapid removal of relatively nitrogen-rich tissues by leaf-grazing invertebrates and may result in the loss of nitrogen relative to carbon and an increase in the C/N ratio[31]. Leaf decomposition depends on the C/N ratio of the leaf tissue with plant material with a low C/N ratio decomposing more quickly[22]. As a general rule, a C/N ratio of approximately 10 is considered optimal for the decomposition of organic matter. The relationship between C/N ratio and percent weight loss by treatment solution in the present experiment is shown in Fig. 3. In the present experiment, the leaf C/N ratio ranged from 15% to 22% for all treatment solutions, which is relatively higher than the optimal C/N ratio range for decomposition. Therefore, no clear relationship between percent weight loss and C/N ratio was observed.

The *Anisocentropus* specimens used in the experiment selectively fed on the epidermal and mesophyll tissues of *Quercus glauca* leaf tissues. In addition, larvae also used *Quercus glauca* leaves for constructing cases. The sum effect of these biological leaf processing behaviors produced FPOM. In the present experiment, however, little FPOM was generated in each chamber, implying either that the generated FPOM was produced by larval eges-

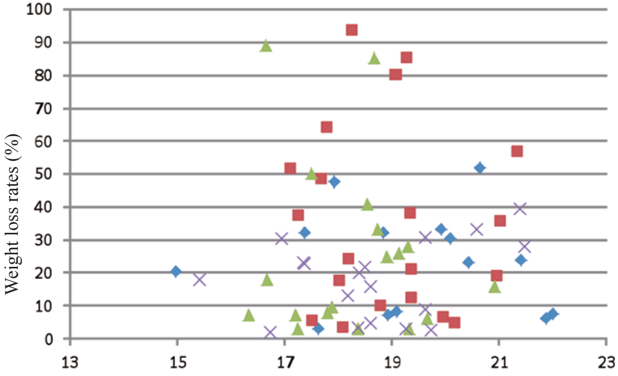

Fig. 3. Relations between percent weight loss and C/N ratio of selected leaves

tion or that the amount of generated FPOM was too small to collect due to the small number of *Anisocentropus* larvae used. Since *Lepidostoma unicolor*, which belongs to case-building shredder as *Anisocentropus*, was reported to consume more than three times its body weight per day[13)], it is possible that the *Anisocentropus* larvae in the present experiment may have been food-limited.

4-3. Utilization of dissolved substances in leachates for growth by *Anisocentropus* larvae

In the present experiment, the percent weight loss for leaves ranged from 16% to 27%, which was significantly higher in the chambers with distilled water ($P < 0.0001$) and lowest in the chambers with leachate2 and nearly equal to those with leachate1. The overall order of initial concentration of TC was leachate1 > stream water > leachate2 > distilled water. Many dissolved substances seem to be generated by natural leaching in side-pools in stream ecosystems. Stream water and leachate solutions (leachate1 and leachate2) are of relatively high quality compared to the leaves, and if dissolved substances are present in sufficient concentration, higher invertebrate growth rates may be supported on dissolved substances than on leaves. In contrast to the high nutritive values of the stream water and leachates, distilled water contains no leachate or natural materials and is markedly less suitable for invertebrate growth and emergence. Major soluble carbon compounds in the treatment solutions are carbohydrates and polyphenol compounds, which are indispensable for invertebrates growth. In the present experiments, therefore, consumption of *Quercus glauca* leaves by *Anisocentropus* larvae was promoted in the solutions with the poorest dissolved nutritive qualities.

Anisocentropus larvae typically grow to at least 15 mm before emerging[15)]. Based on the size of larval cases at the start of the experiments, the medium and large sizes of larvae may be at the pre-terminal or terminal instar stages; however, the data obtained at this time are insufficient to determine the precise life history of *Anisocentropus*. In the present study, larval growth rates were significantly higher in chambers with stream water and leachate1, and lowest larval growth was obtained in the chamber with distilled water. These observations are completely opposite to the results obtained for 30 day submerged leaf processing rates, as described above.

It is, therefore, possible that *Anisocentropus* larvae, which are shredder species, prefer high quality food[3)] and utilize leachate as a high quality food resource instead of *Quercus glauca* leaves, which would give a low assimilation efficiency, except for the chamber with distilled water. In the chamber with distilled water, *Quercus glauca* leaves were the only nutritional resource, and feeding on leaves might have been accelerated.

In the present experiment, the pupation and emergence were relatively high in large larvae. Pupation and emergence of larvae were relatively high in distilled water and relatively low in stream water; however, statistical treatment of the data failed to show significant differences for some comparisons. It is possible that a high-quality food supply controls the *Anisocentropus* life cycle through its influence on metabolism and is measurable as feeding and growth rates such that growth and emergence of *Anisocentropus* larvae are encouraged. *Anisocentropus* larvae may pupate and emerge to escape from the conditions of the distilled-water treatment-groups in the experiment, which deviated from those of natural stream. However, results from our preliminary experiments in which sufficient amounts of leaves were supplied for food produced around 50% pupation plus emergence rates after 30 days of submergence. Therefore, more experiments mimicking natural conditions or field studies are necessary to verify pupation and emergence behaviors of *Anisocentropus* larvae.

Invertebrate growth rate and, to some extent, survivorship, are both affected by food quality and quantity[7)]. Thus, the relatively high growth in chambers with stream water or leachate may be attributed to the fact that both of these chambers contained large amounts of dissolved substances leached from leaves and consequently had high quality food to support robust growth.

4-4. Change in water quality due to feeding and case-building behaviors of *Anisocentropus* larvae

When *Quercus glauca* leaves were submerged in stream water or distilled water, many water-soluble substances began to leach from the leaves, depending mainly on the conditioning rates, initial concentrations and the initial weight of the leaves that were submerged[17, 18)]. Some substances are consumed by microbes and invertebrates that are active in the water[20)].

In the present experiments, TC, TN and cations were leached from *Quercus glauca* leaves in the chambers with stream water and distilled water, corroborating the results of previous studies. (In the present study, anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) could not be measured due to mechanical trouble with the measuring instrument). The release rates of each substance were somewhat different from those from previous studies, possibly due to differences in conditioning rates of *Quercus glauca* and the use of a different invertebrate. *Anisocentropus* larvae were used in the present experiment, compared to *Lepidostoma japonicum* larvae in the previous study.

Several pathways, such as mechanical decomposition of the leaves and associated microbes, excretion by shredders[29)] and leaching from *Quercus glauca* leaves and feces of *Anisocentropus* larvae, as well as feeding and case-building activities, were possibly active in each of the chambers in the present experiment. Some substances were generated and some substances were consumed through this processing. Thus, differences between the initial and final concentrations of each substance, as shown in Table 2, may contribute to clarifying these mechanisms. However, variations in the initial concentrations of TC, TN and cations and the extent of microbial activity in each chamber among treatments make it difficult to make direct comparisons of changes in rates from initial to final concentrations (after 30 days of submergence).

The results of the leaching experiment in which a leaf was divided into several pieces showed that the concentrations of TC, TN and NH_4^+ tended to decrease with the number of piece, but the conditioning rates are considered to differ among the leaves, depending on the number of pieces. The decrease with increasing number of pieces divided in TC is likely a result of biological uptake[27)] induced by microbes attached to the leaves. While increases in nitrogen content are generally thought to be due to increased microbial population[5)], accumulated nitrogen content, such as TN and NH_4^+ during leaf decomposition was not accelerated by the release from leaves due to physically separating the leaves into pieces in this experiment. The reason for this different pattern may be the immobilization of N in microorganisms.

The concentrations of K^+, Mg^{2+} and Ca^{2+} in water tended to increase with an increasing number of pieces. Experimental results with terrestrial ever-green leaves *Eucalyptus delegatensis*31) showed the concentration of K^+ to decrease in a manner that was positively correlated with rainfall, which emphasizes the importance of leaching and suggests that K^+ is water-soluble. Likewise, Mg^{2+} and Ca^{2+} were likely water-soluble and lost through the conditioning of the leaves due to the specific physiochemical conditions. The amount of water-soluble organic substances that leaches from needle litter during one day could be increased from about 1%~10% to 12% if the litter was ground before conditioning26). Thus, it is possible that biological decomposition, such as grazing and case-building behaviors of invertebrates accelerates the leaching processes of water-soluble substances. In the present experiments, water-soluble substances, such as K^+, Mg^{2+} and Ca^{2+} tended to be released more easily in chambers that contained *Quercus glauca* leaves divided into four pieces. The overall size of these pieces was nearly the same as the mean length of the line of apsides of the leaf-disc case of *Anisocentropus* larvae that were regarded as being of a large size in this experiment. Therefore, the influence of case-building behavior of *Anisocentropus* larvae on stream water composition reached a maximum for *Anisocentropus* larvae at the large size. *Quercus glauca* leaves, which have a tough surface consisting of cutin, seem to be suitable for case-building based on our previous study20) ; however, it decomposes more slowly than leaves of other more quickly decomposing species.

5. Conclusion

Food resource categories based on particle size and classification of functional feeding groups among stream invertebrates provide a useful means for describing the morpho-behavior capacity of stream invertebrates that consume available food resources, such as CPOM, FPOM, UPOM and DOM8). The intergrade and distinctions, however, are not always clear and there is a certain number of specializations in leaf-eating invertebrates. In this classification, shredders, such as *Anisocentropus* larvae, have been regarded as consumers of CPOM and transformers CPOM to FPOM, UPOM and DOM through their feeding, digestion and excreting behaviors1,6). In the present experiment, the results indicate that *Anisocentropus* larvae, which are classified as shredders, also utilize dissolved substances (DOM) leached from *Quercus glauca* leaves, which is a relatively high quality food for survivorship. The utilization of leachates as relatively high quality food for shredders was observed in our previous experimental results conducted on *Lepidostoma japonicum* larvae20). Upon entering stream systems, leaves (CPOM) are colonized by fungi and bacteria, which accelerates the conditioning of the leaves. Because most stream macroinvertebrates are omnivores, and shredders usually prefer CPOM that has been well colonized by microorganisms8), and conditioning results in increased palatability to invertebrate consumers13). One possible explanation for the results in this experiment is that the *Quercus glauca* leaves used in the present experiment were not well conditioned for *Anisocentropus* larvae. It is also possible that the pre-terminal and/or terminal larval instars used in the present experiments (large and/or medium sized larvae) require a relatively higher quality protein contained in the leachates because intake of high-protein food is important for a detritivore during the last phase of rapid growth2). The leachates from leaves are of relatively higher quality compared to nutrients derived from the leaves themselves. In addition to the chemical parameters measured in the present study, other dissolved materials are also expected to be present in the leachate.

In the present study, the fragmentation of leaves resulting from feeding and case-building by insect larvae promotes leaching from water-soluble leaf materials. This is supported by previous experimental results using *Lepidostoma japonicum* larvae20). Trichoptera which built nest cases mainly with plant materials, such as *Anisocentropus* and *Lepidostoma japonicum* larvae, prefer lentic conditions with relatively shallow water-depth, such as side-pools, in natural streams. Under the circumstances in the study site, leaves supplied from riparian forests tend to be retained and accumulated in side-pools, leaching many substances from the leaves. These released substances are accumulated in side-pools, accelerating the heterogenic composition of stream water17). *Anisocentropus* and *Lepidostoma japonicum* larvae may contribute to promoting the heterogeneity of stream water composition through consumption of substances in leachates, excretion of feces and release of alternative substances from their feces. However, the importance of shredders for generating dissolved organic compounds that become part of the stream food budget has not been extensively investigated.

The potential effects of currents on leaf decomposition were decreased by conducting the experiments in chambers. In addition, only leaves of *Quercus glauca* and larvae of *Anisocentropus* were used in the present study, and any potential compounding effects of other leaf or invertebrate species were also excluded. Consequently, predation, competition with other shredders, and other effects associated with the natural food supply are excluded in the present experiments. Since the effect of conditioning duration, microbial colonization and growth differ for leaves of different tree species, and because preference for *Quercus glauca* leaves varies among shredder species, field experiments with different combinations of leaf and insect species are required.

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コバントビケラ（*Anisocentropus*）幼中の溶出成分利用の可能性と摂食・営巣行為が渓流水質に及ぼす影響とについて

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要　約

河畔域から渓流に供給されるリーフリターは膨大な量にのぼると推測され，渓流に生息する水生生物の栄養源の大半を占めると言われているが，水生生物のリーフリターの摂食の詳細は摂食機能群と関連付けて論じられる事例はあるものの不明な点も多く，また，水生昆虫幼虫の摂食・営巣行為が渓流水質に及ぼす影響についてはほとんど知られていない．そこで本研究は，南九州渓流に広く生息するコバントビケラ幼虫による溶出成分の利用の可能性と，摂食・営巣行為がアラカシリーフリターからの成分溶出に及ぼす影響を，実験的に明らかにすることを目的とした．

2014年3月，一ツ瀬川支流竹尾川下流（宮崎県西都市）の河畔域に優占するアラカシ500枚，ヤマビワ100枚，エゴノキ100枚の葉（落葉直前のもの）を採取し，十分な洗浄の後，10Lの蒸留水中に30日間投入し溶出成分を抽出し溶出水とした．さらに，この実験で使用したアラカシの葉500枚を，十分に洗浄・乾燥し重量を測定した後，3個×4組の水槽（25×15×10cm）に上記のアラカシの葉6枚づつと，別途現地で採取したコバントビケラ幼虫３匹づつを投入し（コバントビケラは，巣の全長に応じて，15mm以下，16～25mm，26mm以上のものに区分し水槽毎に投入した），それぞれ500mlの渓流水，蒸留水，溶出水，希釈溶出水（濃度を1/2に希釈した溶出水）で満たした．本実験では，各組5回づつ繰り返しを設けサブサンプルとした（合計60サンプル）．各サブサンプルは市販ポンプにより酸素を供給し温度調整をしない実験室に置いた．30日後，アラカシリーフリターの乾燥重量を計測し，コバントビケラの成長率，羽化率，死亡率を測定するとともに，投入水に含まれる全炭素量（TC），全窒素量（TN），陽イオン濃度を測定した．また，コバントビケラの摂食・営巣活動に伴うアラカシリーフリターの断片化が成分溶出に及ぼす影響を確認するために，アラカシリーフリターをさまざまな大きさに分割して蒸留水を満たした水槽に投入し，30日後の上記成分濃度を測定した．

溶出とコバントビケラ幼虫の摂食・営巣行為に伴う30日間でのアラカシリーフリターの重量損失は16〜27 %であり，蒸留水で最大，希釈溶出水で最小を示し，溶出水でのアラカシ重量損失は希釈溶出水とほぼ同じであった（$P<0.0001$）．これに対して，コバントビケラの成長率は蒸留水で最小となった（$P<0.0001$）．さらに，アラカシリーフリターを1/8（コバントビケラの巣に匹敵する大きさ）に分割した場合に，K^+，Mg^{2+}，Ca^{2+} 等の水溶性溶存成分の溶出が進行することが確認された．

以上の実験結果から，これまでデトリタス食者として位置づけられ，リーフリターの分解過程においてCPOMをFPOM，UPOM，DOMに転換する役割を担うとみなされていたコバントビケラ幼虫は，より質の高い栄養源として溶出成分（DOM）も利用している可能性があり，摂食・営巣行為に伴う断片化による成分溶出の促進を通じて，渓流水質の成分組成に影響を及ぼしていることが示唆された．