Effect of leaf-decomposition by *Lepidostoma japonicum* larvae on stream water physicochemistry

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To identify leaching characteristics from initial decomposition of evergreen *Quercus glauca* leaf litter in relation to leaf-decomposition behavior of invertebrate larvae, the potential utilization of leachates from conditioned *Quercus glauca* leaves (which are colonized by microbes) by larvae of *Lepidostoma japonicum* and the effects of leachates on stream water quality during initial leaf decomposition were examined.

Leaves collected at the 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 at 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 decomposition rate of leaves, EC, pH, TC(total carbon), TN(total nitrogen), Na^+, NH_4^+, K^+, $Cl^-$and NO_3^- ($P<0.005$). 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.

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

1. INTRODUCTION

Allochthonous material, such as leaves, is the main energy source for consumers in small streams. Organic matter is decomposed by a variety of physical, chemical and biological processes1). After leaves enter streams, soluble chemicals are leached from plant tissues, which are also colonized by microbes and fragmented by mechanical abrasion and invertebrate activity. The instream processing of this organic material produces dissolved organic matter (DOM)25) and inorganic nutrients8, 26). Since considerable quantities of allochthonous material enter lotic ecosystems from riparian forests6), substances derived from the decomposition of leaf litter are considered to have an important effect on stream water quality. As allochthonous organic material provides 50-90% of the energy transformed by stream organisms10), decomposed leaves are therefore an important source of organic carbon in small forest streams.

The importance of allochthonous material as an energy source for lotic organisms has been examined extensively. Herbst, G.N.(1982) has focused on the fate of allochthonous material, principally leaves, entering streams12). In addition, the importance of allochthonous organic matter in streams has been demonstrated in dicades and the functioning of forest stream ecosystems is dependent upon the considerable contribution of dead leaves from riparian vegetation3).

During the initial decomposition of leaves, the saprophytic fungi and bacteria that colonize the leaves form a protein-rich biofilm on the leaf surface. These leaves, which are referred to as conditioned leaves10), have been shown to promote invertebrate feeding. Conditioning is thus an important microbial process that increases the palatability of leaves for consumption by invertebrate consumers. This increased palatability may be attributed to an increase in the softness of the leaf tissue over time[10].

Several studies have examined microbial immobilization of organic carbon, phosphorus and nitrogen derived from decomposing plant material[26, 30]. The dependence of woodland stream ecosystems on allochthonous organic matter, primarily in the form of autumn-shed leaf litter, is well documented. However, few studies have examined the chemical constituents of leachates derived from evergreen leaf litter, or their effect on stream water quality during initial decomposition, conditioning, and subsequent degradation of leaves by the feeding behaviors of leaf-shredding aquatic insects.

We previously showed that the decrease in the weight of evergreen *Quercus glauca* Thunb. (It is denoted as *Quercus glauca* from now on in this study) leaves submerged in water for more than 30 days was less than 25% of that from deciduous leaves, and that *Quercus glauca* leaves released soluble leachates, such as TC and TN, as well as the cations K^+, Mg^{2+} and Ca^{2+}[22]. In addition, we also showed that the tough outer surfaces of evergreen leaves (e.g., cuticle) may delay leaching and subsequent weight loss in leaves until the outer layer of the epidermis, containing cutin, degrades in water. For example, *Quercus glauca* leaf litter is one of the primary sources of soluble leachates in stream water, particularly in lentic river reaches such as pools and side-pools, resulting in a heterogeneous distribution of leachates over the length of a stream[20].

In this study, the potential utilization of leachates from conditioned *Quercus glauca* leaves by larvae of the leaf-shredding caddisfly, *Lepidostoma japonicum*[17], was examined. *Lepidostoma japonicum* is dominant in lentic stream environments that are characterized as a heterogeneous distribution of leachates. In addition, we also examined the effects of leachates on stream water quality during initial leaf decomposition, as well as during conditioning and subsequent processing by aquatic insects.

2. METHODS

2-1. Site description

Experiments were conducted in the Takeo River in Saito City of Miyazaki Prefecture. The Takeo River, a tributary of the Hitotsuse River, ranges in elevation from 100 m to 600 m and originates in the Southern Kyusyu 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 ranging from 20 - 30 cm/sec. The stream bottom is composed primarily of gravel, pebbles and cobble substrates and consists of a series of riffles, pools, and side-pools with irregular meanders.

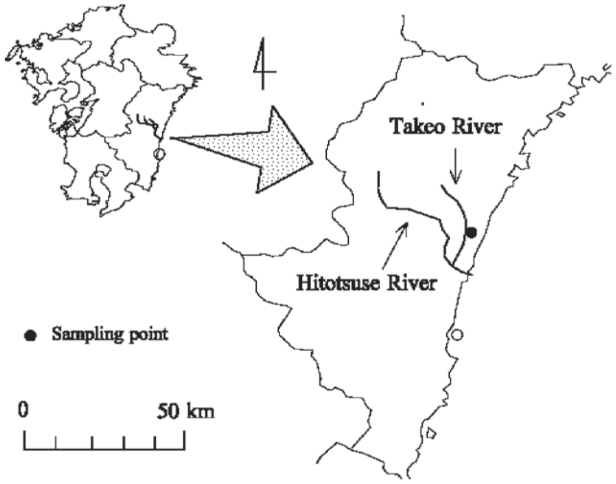


Fig. 1. Investigated site.

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 to the forest floor tend to be transported to the stream.

The invertebrate fauna of the Takeo River is rich and over 50 taxa have been recorded to date and during the winter and spring, *Lepidostoma japonicum* is the most abundant aquatic invertebrate in the Takeo River[19]. Larvae most commonly are found among deposits of allochthonous organic material in lentic areas such as pools and side-pools in the stream. *Lepidostoma japonicum* larvae, which construct cases composed of leaf materials, typically grow to at least 15 mm before emerging from May to October[17]. However, the data obtained at this time are insufficient to indicate the precise life histories of the *Lepidostoma japonicum*. *Lepidostoma japonicum* belongs to the family Lepidostomatidae, which contains shredder detritivores that feed upon large particulate leaf material and use leaves for case-building and habitat[27].

2-2. Materials

The materials used in the present study included leaves and larvae from the dominant plant and leaf-shredding macroinvertebrate species, *Quercus glauca* and *Lepidostoma japonicum*, respectively. All of the specimens were obtained from along the investigated reach.

A total of 500 leaves were collected from the branches of a single *Quercus glauca* tree 2 to 4 m above the ground at the study site on March 19, 2013. The tree had been growing in the riparian zone of an exposed, unfertilized section of river flowing over an alluvial plain. All leaves were sun old-leaves that had been picked from the old branches of the tree just before abscission; leaf emergence of *Quercus glauca* at the study site started in the 4th week of March in 2013, and expansion of leaves occurred from April to May. The whole leaves collected retained their original shape without any skeletonization. Stream water (100 L) was also collected from the same stream for leaching experiments.

Larvae of *Lepidostoma japonicum* were most common in spring in side-pools of the river section being studied. Larvae were typically found in allochthonous organic material that had been deposited on course to fine sand in slow-flowing water. Total of 200 larvae were collected on March 26, 2013, and an attempt was made to select macroinvertebrates that were uniform in size. Specimens were maintained in aerated water at ambient temperature (20°C) for five days to prevent larvae from being feeble before starting the experiments.

2-3. Laboratory experiments

Collected leaves were washed with distilled water to remove aerosol dust and atmospheric gases, air-dried in air for 1 week and then oven-dried at 80°C for 12 hr. Chambers (40cm × 15cm × 20cm) were filled with 1000 ml of stream water, and 1, 3, 6, 12, and 24 randomly selected leaves were then weighed and placed into each of the five chambers, respectively. Two series of chambers were prepared in this way; one series for the experimental treatment (experimental chambers: Ae-Ee) and the other for the control treatment (control chambers: Ac-Ec). Further, each series had five replicates (sub-samples), which meant that there were a total of 25 experimental chambers, and 25 control chambers. The water in each chamber was aerated to simulate the physical environment of leaf litter in the side-pools of the stream being investigated. Since the cuticle of *Quercus glauca* leaves is a physical obstacle to invertebrate feeding, the leaves were incubated for 10 days to degrade the cuticle and promote conditioning and leaching[22)]. Ten days after leaf immersion, the wet length of *Lepidostoma japonicum* larval cases was measured and six similar-sized larvae were placed in each of the experimental chambers (i.e. chambers Ae-Ee). Animals were checked daily and the experiment was terminated if they 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 remaining materials were separated into processed leaves and leaf fragments $\geqq 1$ mm in diameter, or coarse particulate organic matter (CPOM), and leaf fragments and particles which $<$ 1 mm in diameter, fine particulate organic matter (FPOM) using a sieve. The CPOM and FPOM in each sub-sample were air-dried for 1 week, oven-dried at 80°C for 12 hr, and weighed. Larval case lengths were measured and weighed after oven-dried at 80°C for 12 hr. Each leachate sample was filtered through 0.20 μm filters just before measurements.

The water physicochemical parameters of each subsample were also measured. Electric conductivity (EC) and hydrogen ion concentration (pH) of the water were measured using a water quality probe (WQC-20A, TOA Electronics Ltd., Japan). Cations (Li^+, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) and anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) in the water were measured by ion chromatography (DX-120, Nippon Dionex K.K., Japan). The concentration of total carbon (TC; including total organic carbon (TOC) and inorganic carbon (IC)) and total nitrogen (TN) were measured with a TOC-TN analyzer (TNC-6000, Toray Engineering K.K., Japan).

3. RESULTS

3-1. Weight loss of leaves after submergence by decomposition

In the present experiments, no significant differences were observed between the control and experimental treatments in the initial weight of the leaves ($P > 0.1$).

Weight loss was calculated by subtracting the final weight of the leaves after submergence from the initial weight, and was evaluated as percentage {((initial weight)-(final weight))×100/(initial weight))} in each control and experimental chamber. The mean weights and standard errors in each treatment are shown in Table 1. In the control and experimental conditions, the change in weight of leaf processing increased with an increase in the amount of leaves. However, percentage processing of the leaves decreased in chambers containing more leaves, because the total amount of leaf material exceeded the processing rate of the leaves in each chamber. The percentage processing in the control Cc sample containing 6 leaves (20.82%) was similar to that observed in our previous study using 5 leaves (24%)[22)].

Since the *Lepidostoma japonicum* larvae used the leaves for food, case construction, and microhabitat, the change of weight and percentage processing of leaves was approximately two-fold greater in the experimental samples than in the control. The overall percentage processing due to feeding and case-building by *Lepidostoma japonicum* could therefore be estimated by subtracting the percentage processing of the control chamber from that of the experimental chamber in each treatment. The overall processing rates of 6 *Lepidostoma japonicum* larvae over 30 days was therefore estimated to range from 27 to 50% of the initial leaf weight.

The biological conversion of leaf litter to FPOM occurs primarily by two mechanisms: maceration by aquatic hyphomycetous fungi, and consumption and egestion by shredders[31)]. Thus, it is considered that the FPOM col-

Table 1. Comparison of leaf weight-loss rates and FPOM production for each treatment

mean ± standard error

		experimental conditions			the weight of leaves				
treatments	chambers	water (ml)	leaves	insects	initial (g)	final (g)	weight loss(g)	percentage (%)	FPOM (mg)
					①	②	①−②	(①−②)* 100/ ①	
control	Ac	1000	1	0	0.49 ± 0.08	0.36 ± 0.06	0.14 ± 0.02	27.46 ± 0.73	−
	Bc	1000	3	0	0.93 ± 0.08	0.68 ± 0.07	0.25 ± 0.02	27.36 ± 0.98	−
	Cc	1000	6	0	2.58 ± 0.11	2.05 ± 0.09	0.54 ± 0.02	20.82 ± 0.13	−
	Dc	1000	12	0	4.06 ± 0.14	3.36 ± 0.12	0.70 ± 0.04	17.21 ± 0.68	−
	Ec	1000	24	0	7.41 ± 0.18	6.31 ± 0.24	1.10 ± 0.11	14.95 ± 1.65	−
experimenral	Ae	1000	1	6	0.55 ± 0.04	0.12 ± 0.02	0.43 ± 0.03	77.06 ± 3.11	179 ± 33
	Be	1000	3	6	0.79 ± 0.02	0.20 ± 0.01	0.56 ± 0.03	71.75 ± 2.99	142 ± 13
	Ce	1000	6	6	1.86 ± 0.08	0.95 ± 0.05	0.95 ± 0.03	49.66 ± 1.00	138 ± 13
	De	1000	12	6	3.60 ± 0.04	1.99 ± 0.01	1.58 ± 0.04	44.59 ± 1.09	129 ± 14
	Ee	1000	24	6	7.49 ± 0.16	4.95 ± 0.13	2.55 ± 0.07	33.99 ± 0.30	391 ± 91

Table 2. Results of physiochemical analysis

mean ± standard error

treatments	chambers	experimental conditions			physiocemicalparameters	
		water (ml)	leaves	insects	EC (μS/cm)	pH
control	Ac	1000	1	0	64.25 ± 1.29	7.1 ± 0.1
	Bc	1000	3	0	61.06 ± 0.98	6.9 ± 0.1
	Cc	1000	6	0	79.02 ± 1.77	6.8 ± 0.1
	Dc	1000	12	0	94.95 ± 1.84	7.1 ± 0.1
	Ec	1000	24	0	130.76 ± 1.21	7.0 ± 0.0
experimenral	Ae	1000	1	6	79.15 ± 1.13	7.8 ± 0.1
	Be	1000	3	6	69.75 ± 2.16	7.7 ± 0.1
	Ce	1000	6	6	75.74 ± 1.77	7.7 ± 0.0
	De	1000	12	6	81.26 ± 0.83	7.6 ± 0.1
	Ee	1000	24	6	94.72 ± 0.99	7.6 ± 0.1

lected in each chamber was generated by egested particles and mechanical fragmentation of the leaf during feeding by *Lepidostoma japonicum* larvae. Because of their small size, individual *Lepidostoma japonicum* larvae produced relatively little feces. Consequently, FPOM was considered to consist of material derived from both feces and mechanical fragmentation. Total FPOM production over the 30-day study period was approximately 100-400 mg (Table 1).

3-2. Leaching characteristics of *Quercus glauca*

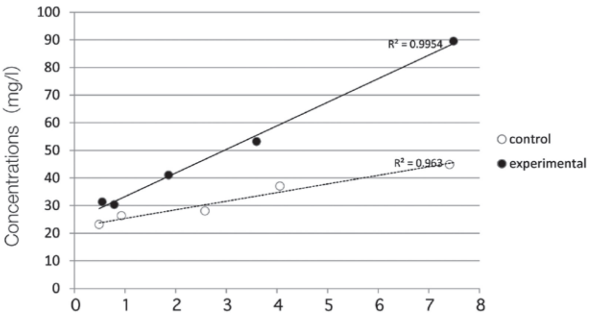
3-2-1. Changes in EC and pH values of water from submerged leaves

The changes in pH and EC of the stream water after the 30-day experimental period are shown in Table 2. The initial EC and pH of stream water was 65.0 μS/cm and 7.0, respectively.

The EC increased over time in all water samples and marked differences were observed after 30 days. While changes in EC in chambers containing fewer than 12 leaves was slight, marked increases in EC were observed in chambers containing 12 and 24 leaves, indicating that some conductive materials leached from submerged leaves immediately after submergence. The mean EC values on the 30th day of the experiment were lower in the experimental chambers than in the control chambers, except for chambers containing fewer than 12 leaves. It may indicate that some conductive materials were consumed by the larvae in these chambers. Compared to immersion experiments using leaves from deciduous broadleaved trees[13, 18, 32], the increase in EC values associated with leaching from *Quercus glauca* leaves tends to be slower due to the relatively thicker epidermal layer of *Quercus glauca*.

The mean pH values decreased after submersion in the control chambers and increased in the experimental chambers. Unlike EC, no marked differences were observed in pH after 30 days of submergence among treatments, even though numerous complex interactions between pH and nutrient cycles were observed; the effect of EC and pH on accelerating leaf decomposition in aquatic ecosystems has been explained elsewhere[14, 15].

(a) TC



(b) TN

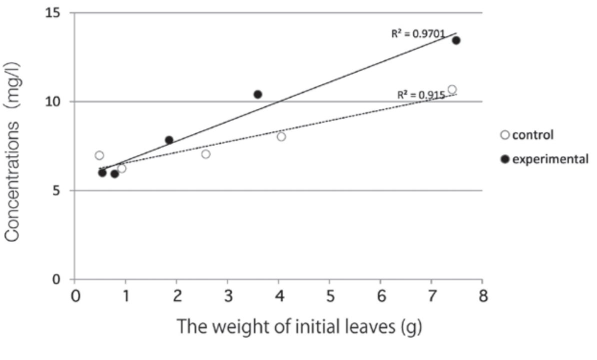


Fig. 2. Mean concentrations of (a) TC and (b) TN in experimental and control water samples relative to initial leaf weight.

3-2-2. Total carbon and total nitrogen leached from leaves

Figure 2 shows the changes in mean TC and TN concentrations of the water relative to the initial weight of leaves in the control and experimental chambers after 30 days of submersion. The initial TC and TN concentrations in stream water were 7.90 mg/l and 1.36 mg/l, respectively. The ranges of standard error were from 0.30 to 2.50 mg in TC and from 0.05 to 0.32 in TN. In the present study, because no IC was detected in any of the samples,

Table 3. Final ion concentrations after 30 days incubation

mean ± standard error (mg/l)

		Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO^{2-}	Br^-	NO_3^-	SO_4^{2-}
control	Ac	0.98 ± 0.01	5.88 ± 0.18	1.28 ± 0.16	2.20 ± 0.05	3.34 ± 0.07	4.36 ± 0.14	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	4.04 ± 1.35
	Bc	1.05 ± 0.01	4.17 ± 0.15	2.31 ± 0.09	2.08 ± 0.01	3.17 ± 0.06	5.41 ± 0.08	0.15 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	6.64 ± 0.18
	Cc	1.15 ± 0.01	5.15 ± 0.27	5.43 ± 0.34	2.44 ± 0.02	2.92 ± 0.01	8.04 ± 0.25	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	5.96 ± 0.12
	Dc	1.18 ± 0.03	6.39 ± 0.05	8.91 ± 0.34	2.72 ± 0.06	2.95 ± 0.04	10.90 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.08 ± 0.20
	Ec	1.26 ± 0.02	6.48 ± 0.34	15.58 ± 0.15	3.53 ± 0.05	3.26 ± 0.05	16.59 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.36 ± 0.09
experimental	Ae	1.21 ± 0.33	3.56 ± 0.95	2.50 ± 0.88	2.31 ± 0.82	4.91 ± 1.42	2.49 ± 1.23	0.00 ± 0.00	0.00 ± 0.00	0.34 ± 0.17	4.80 ± 1.98
	Be	1.37 ± 0.41	3.94 ± 0.99	2.40 ± 0.84	2.51 ± 0.68	3.88 ± 1.41	4.43 ± 1.24	0.17 ± 0.04	0.00 ± 0.00	0.21 ± 0.09	5.05 ± 1.74
	Ce	1.78 ± 0.04	7.31 ± 0.14	5.93 ± 0.17	3.61 ± 0.19	4.94 ± 0.23	6.07 ± 0.41	0.15 ± 0.04	0.00 ± 0.00	0.15 ± 0.05	7.91 ± 0.52
	De	1.52 ± 0.04	10.65 ± 0.13	10.10 ± 0.41	3.40 ± 0.13	4.17 ± 0.23	5.90 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.04	7.07 ± 0.09
	Ee	1.54 ± 0.01	12.21 ± 0.13	17.16 ± 1.05	2.58 ± 0.23	2.75 ± 0.19	5.17 ± 1.18	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.05	3.17 ± 0.99
stream		0.90	0.99	0.98	2.40	3.24	2.20	0.69	1.35	2.91	4.30

TC concentration was equal to TOC concentration. TC and TN are typically correlated with initial leaf weight, and high correlation coefficients (>0.9) were obtained for all treatments. Leaching rates of TC and TN were higher in experimental chambers than in control chambers.

3-2-3. Ion concentrations leached from submerged leaves

Table 3 shows the concentrations of cations and anions leached from leaves submerged in stream water. Li^+, F^- and PO_4^{3-} were not detected in all samples during the experimental period. However, Br^- which was detected at low levels in stream water, disappeared in each treatment water. Except for Br^-, NO_2^- and NO_3^-, the ion concentrations were higher in each treatment water than in stream water, indicating that these ions were supplied from leaves by leaching.

The concentrations of Cl^- tended to increase with an increase in the quantity of submerged leaves in the control chambers, while Na^+, NH_4^+ and K^+ increased with the amount of leaves in experimental chambers. The concentrations of Mg^{2+}, Ca^{2+}, and SO_4^{2-} did not fluctuate in accordance with the quantity of leaves in each chamber. The Na^+, NH_4^+, and K^+ concentrations were higher in the experimental chambers than in the control chambers, particularly in those chambers with 12 and 24 leaves. The concentrations of NO_2^- and NO_3^- were also higher in experimental chambers and were not detected in control chambers. Cl^- concentrations were higher in all of the control chambers in each treatment. Among the ions, NH_4^+, K^+, and Cl^- were leached most abundantly, with levels being one order of magnitude higher than those of the other ions in chambers with 12 and 24 leaves.

3-3. Growth and emergence rate and mortality of *Lepidostoma japonicum* larvae

Increases in the case length and size of *Lepidostoma japonicum* larvae, as well as rates of larval emergence and mortality in each experimental chamber over the course of the experiment are summarized in Table 4. *Lepidostoma japonicum* larvae used *Quercus glauca* leaves for food, case-building, and habitat in the experiments. After 20 days, some of the larvae in chambers with 6 to 24 leaves grasped onto stationary leaves or sank to the bottom of chambers before emerging.

As shown in Table 4, the range in the mean length of larval cases after the experiment ranged from 8.2±0.7 to 10.4±0.5 mm, however, that before the experiment was from 7.1±0.4 to 7.8±0.5 mm. The growth rate of case which defined as (final size)/(initial size) in this paper was highest in chamber De, which contained 12 leaves, and was lowest in chamber Ae, which had one leaf; however, a significant correlation was not observed in the growth rate among chambers ($P>0.05$). The rate of larval emergence tended to be high in chambers containing more leaves and was highest in chamber De. No larvae emerged from chambers Ae and Be, which contained 1 and 3 leaves, respectively. Larval mortality tended to be low in chambers containing more leaves, with chamber Ee, which contained the maximum number of leaves, having zero mortality. Chamber Be, containing 3 leaves, had the highest mortality with 33 % deaths. Significant correlations were observed between the rate of emergence and mortality in all chambers ($P<0.005$).

Because the ingestion of some *Lepidostoma japonicum* cases by other *Lepidostoma japonicum* individuals may have had the effect of decreasing the number of cases in the chambers, in chambers containing fewer leaves, it was expected that considerable damage to cases would occur as a result of there being relatively little food; however, larvae were never observed eating their own or other cases during the experimental period. In addition, larval cases did not appear to be damaged and no naked larvae were observed.

Table 4. Growth rates, emergence rates, and mortalities of *Lepidostoma japonicum* larvae after 30 days in experimental chambers

mean±standard error

	case sizes of larvae(mm)		growth rates	emergence rates (mean(%))	mortalities (mean(%))
	initial ①	final ②	②／①		
Ae	7.1 ± 0.4	8.2 ± 0.7	1.24 ± 0.08	0	22
Be	7.8 ± 0.5	9.3 ± 0.4	1.39 ± 0.22	0	33
Ce	7.7 ± 0.3	9.9 ± 0.4	1.34 ± 0.09	22	28
De	7.4 ± 0.2	10.3 ± 0.2	1.50 ± 0.08	56	6
Ee	7.7 ± 0.1	10.4 ± 0.5	1.39 ± 0.11	33	0

Growth rates were defined as (final size)/(initial size) in this paper.

3-4. Two-way ANOVA analysis

Table 5 shows the results of two-way analysis of variance. In this analysis, treatments (control and experimental) and the number of submerged leaves in each chamber were regarded as independent variables and the measured values were considered as the dependent variables. Significant differences between control and experimental treatments were observed in decompositopn rate of leaves, EC, pH, TC, and TN ($P<0.001$); however, significant interactions were observed between each variable pair. Among the ions assayed, significant differences were observed between the control and experimental treatments in Na^+ ($P<0.005$), NH_4^+ ($P<0.001$), K^+ ($P<0.05$), Cl^- ($P<0.001$) and NO_3^- ($P<0.001$) and significant interactions were observed in NH_4^+, NO_3^- and Cl^-.

4. DISCUSSION

4-1. Leaf processing by *Lepidostoma japonicum* larvae

In deciduous leaves, weight loss by leaching occurs within a few days of submergence. For example, autumn-shade leaves in water can lose up to 40% of their dry weight (dw) in a few days[16)]. Similarly, the ash-free dry weight of sugar maple, yellow birch, and beech was 85.4% of the initial weight after 2 days of submergence in the laboratory[27)]. Webster *et al.*[32)] 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.

In the present study, weight loss in evergreen leaves from *Quercus glauca* after 30 days of submergence in

Table 5. ANOVA of all physicochemical parameters in control and experimental treatments

	< decomposition rate >				< EC >				< pH >			
	n	mean square	F	*P*	n	mean square	F	*P*	n	mean square	F	*P*
treatments	1	14322.969	4.085	< 0.001	1	432.862	4.085	< 0.001	1	5.827	4.085	< 0.001
initial weight of leaves	4	1441.899	2.606	< 0.001	4	3448.392	2.606	< 0.001	4	0.070	2.606	< 0.001
interaction	4	403.346	2.606	< 0.001	4	1013.403	2.606	< 0.001	4	0.032	2.606	< 0.001
residual error	40	12.778			40	10.683			40	0.004		

	< TC >				< TN >			
	n	mean square	F	*P*	n	mean square	F	*P*
treatments	1	4764.872	4.085	< 0.001	1	10.826	4.085	< 0.001
initial weight of leaves	4	2681.337	2.606	< 0.001	4	59.926	2.606	< 0.001
interaction	4	675.206	2.606	< 0.001	4	6.672	2.606	< 0.001
residual error	40	10.638			40	0.220		

	< Na^+ >				< NH_4^+ >				< K^+ >			
	n	mean square	F	*P*	n	mean square	F	*P*	n	mean square	F	*P*
treatments	1	1.631	4.085	< 0.005	1	46.067	4.085	< 0.001	1	10.372	4.085	< 0.05
initial weight of leaves	4	0.230	2.606	n.s.	4	53.356	2.606	< 0.001	4	358.348	2.606	< 0.001
interaction	4	0.063	2.606	n.s.	4	26.628	2.606	< 0.001	4	0.920	2.606	n.s.
residual error	40	0.140			40	1.090			40	1.527		

	< Ca^{2+} >				< Mg^{2+} >				< Cl^- >			
	n	mean square	F	*P*	n	mean square	F	*P*	n	mean square	F	*P*
treatments	1	1.043	4.085	n.s.	1	12.582	4.085	< 0.05	1	225.297	4.085	< 0.001
initial weight of leaves	4	1.783	2.606	< 0.05	4	1.847	2.606	n.s.	4	85.227	2.606	< 0.001
interaction	4	1.571	2.606	n.s.	4	2.349	2.606	n.s.	4	45.955	2.606	< 0.001
residual error	40	0.623			40	2.088			40	2.449		

	< NO_2^- >				< NO_3^- >				< SO_4^{2-} >			
	n	mean square	F	*P*	n	mean square	F	*P*	n	mean square	F	*P*
treatments	1	0.007	4.085	n.s.	1	0.399	4.085	< 0.001	1	12.122	4.085	n.s.
initial weight of leaves	4	0.048	2.606	< 0.001	4	0.025	2.606	n.s.	4	33.402	2.606	< 0.001
interaction	4	0.009	2.606	< 0.01	4	0.025	2.606	n.s.	4	5.825	2.606	n.s.
residual error	40	0.002			40	0.022			40	5.072		

control treatment was at 14.95-27.46 % and the percentage processing in control chamber Cc, which contained 6 leaves (20.83%), was similar to the 24% that we reported previously using 5 leaves[22].

In this study, weight loss in leaves was broadly defined as leaching and larval-induced leaf weight loss. It is, therefore, attributed to both assimilated and non-assimilated leaf material, as well as the leachates released from leaves. In the experimental chambers of this study, the percentage processing ranged from 33.99 to 77.06 (%), which was significantly greater than that observed in the control chambers ($P<0.001$). The overall net percentage processing by *Lepidostoma japonicum* larvae, therefore, was approximately 23-50%, if the percentage processing of the control chambers was subtracted from percentage processing of the experimental chambers in each treatment. However, approximately 8 to 10% of weight loss is considered to be due to microbial processing[12]. It is therefore reasonable to assume that the percentage processing of *Quercus glauca* leaf litter by *Lepidostoma japonicum* larvae in each treatment was somewhat lower than that shown in Table 1.

The percentage processing tended to be lower in chambers with fewer leaves, especially in chambers Ae and Be, which contained 1 leaf and 6 leaves, respectively, showing percentage processing more than 70%. Since *Lepidostoma unicolor* was reported to consume more than three times its body weight per day[2], it is possible that the *Lepidostoma japonicum* in chambers Ae and Be may not have had enough food. The minimum percentage processing in the experimental chambers was$<$35% in chamber Ee, implying that the number of leaves may have been sufficient in those chambers. The results of this study corroborate *on site* observations. Specifically, the distribution and abundance of *Lepidostoma japonicum* larvae at the study site was positively related to the presence of conditioned leaves, which are used by the larvae for food and protection.

Lepidostoma japonicum larvae typically grow to at least 15 mm before emerging from May to October[17]. Based on the size of larval cases before starting the experiments, all of the larvae may be at the pre-terminal or terminal instar stage, however, the data obtained at this time are insufficient to indicate the precise life histories of the *Lepidostoma japonicum*. In the present study, rates of larval growth and emergence increased and mortality decreased in chambers De and Ee, which contained 12 and 24 *Quercus glauca* leaves, respectively. Invertebrate growth rate, and to some extent survivorship, are both affected by food quality and quantity[6]. In addition, the growth rates defined as (final size)/(initial size) in this paper of shredders (e.g. *Tipula* spp and *Pycnopsyche* spp) have been reported to range from 0.47 to 1.53, depending upon density[5]. Thus, the relatively high growth and emergence rates, combined with the relatively low mortalities, in chambers De and Ee may be attributed to the fact that both of these chambers contained large amounts of leaf material. It is likely that the abundance of food facilitated the growth and subsequent emergence of the larvae in these chambers.

The *Lepidostoma japonicum* specimens used in the experiment fed on cuticle, epidermal and mesophyll tissues of *Quercus glauca* leaf tissue selectively. In addition, larvae also used *Quercus glauca* leaves for constructing cases. The cumulative effect of these different biological leaf processing behaviors produced FPOM, which was also produced by larval egestion. Total FPOM production by six larvae ranged between 129±14 mg (dw) in chamber De and 391±91 mg (dw) in chamber Ee over a 30-day period, which corresponded to 2.8% and 1.9% of the initial dry weight of leaves. It is possible that relatively large amounts of FPOM were produced, both through egestion and mechanical degradation of leaves, by feeding in chamber Ee because six larvae survived for 30 days without emerging or dying, producing feces and fragments of leaf material.

4-2. Change in water quality composition due to leaf decomposition

TC and TN concentrations leached from leaves tend to be positively correlated with initial carbon and nitrogen contents of the leaves, respectively[23].

In the present experiments, TC was leached from *Quercus glauca* leaves in each chamber, corroborating our previous studies[21,22]. Since the amount of TC that is leached depends on the extent of leaf conditioning, which in turn is affected by the extent of leaching that occurs in the terrestrial environment prior to immersion in water[6], the rates of TC leached in the present experiments were not comparable to those reported previously. Carbohydrates and polyphenol compounds are both considered to be major soluble carbon compounds that are lost from leaves during leaching[30], while lignin, cellulose, and hemicelluloses are considered to remain in leaves after leaching[6].

In deciduous leaves, such as hickory and oak, carbohydrate concentrations decreased very rapidly during the first two weeks of immersion in water, with 70% of the reducing sugars lost from hickory and oak[30] by leaching. These differences in weight loss patterns and release rates of TC between deciduous and evergreen leaves arise from differences in leaf composition and structure. TC is derived from soluble carbohydrates, organic acids and other organic material[30]. Soluble carbohydrates are lost through the cuticle on the surface of evergreen leaves, and the thick cuticle of evergreen leaves reduces the rate of decomposition[22]. In addition, the rate of decomposition of leaf litter in water may be increased by the abrasion of leaf surfaces. For example, the leaf cuticle is composed largely of lipids, which are lost more rapidly than total mass[2]. Broadleaf evergreen leaves, such as those of *Quercus glauca*, however, break down slowly.

In general, feces produced by the larvae of aquatic invertebrates contains less carbohydrates and more protein than their food[31]. As a result, feces were not considered to be a source of TC in this study. However, in the present experiments using pre-terminal and terminal larval instars of *Lepidostoma japonicum*, the TC concentrations that were released in each treatment were higher in experimental chambers than it was in the control chambers (refer to Fig. 2). The reason for this is due to leaching of water-soluble organic substances from leaves that were fragmented due to shredding and case-building by *Lepidostoma japonicum* larvae, because the amount of water-soluble organic substances leached from litter increases from about 1% to 10-12% per day if the litter is ground beforehand[14].

TN concentration also increased in each treatment chamber depending on the number of leaves. The relatively high nitrogen content of decomposing plant material is generally considered to be due to increased microbial populations[4]. This is because some fungi can increase the protein content of the leaves and because the addition of N to the water accelerates fungal growth[10]. In each of the treatments in this study, water TN concentrations were higher in experimental chambers than in control chambers because of nitrogen leaching from the feces produced by the larvae.

In the present study, significant differences were observed in Na^+, NH_4^+, K^+, Ca^{2+}, Cl^-, and NO_3^- concentrations between control and experimental chambers, and also between TC and TN concentrations in both treatments. The increases in NH_4^+ and NO_3^- concentrations in the experimental chambers were due to an increase in TN being leached from the leaves, as described above. Using insect-free leaching experiments, we previously demonstrated that K^+ and Ca^{2+} are leached from *Quercus glauca* leaves when submersed in water for 30 days20, 21, 22). In the present study, the quantities of K^+ and Ca^{2+} that were leached were somewhat lower than those reported previously, likely because of the leaf consumption by *Lepidostoma japonicum* larvae.

The quantity of K^+ leached in the present study was one order of magnitude higher than the other ion concentrations. However, in contrast to the high K^+ ratios in the laboratory water samples, the initial K^+ concentrations in the stream water was lower than the other cations and anions assayed. The lower concentration of K^+ in the stream water would suggest that there is a differential utilization of K^+ by stream biota, and that stream sediments are important for removing leachates from water24). Biological consumption may occur in PO_4^{3-}, which was not detected in the stream water. In the present study, the number of *Lepidostoma japonicum* larvae used may also have been insufficient for reducing K^+ concentrations remarkably by consumption as compared to the amount of leaves, especially in chamber De and Ee.

Of the ions assayed, the concentration of Cl^- was lower in the experimental chambers than in controls, suggesting that Cl^- is utilized in some way in the experimental chambers. In previous studies, Cl^- typically bonded to Na^+. Most of the Na^+ present in stream water is derived from rock weathering, but some Na^+ and Cl^- may be derived from rainwater. Closer to the coast as the watershed of the investigated stream, rainwater may contribute significantly to the supply of ions. However, the reason for the utilization or disappearance of Cl^- observed in the present experiment remains unknown.

4-3. Possible utilization of leachates by *Lepidostoma japonicum* larvae

When leaves fall into streams, soluble organic and inorganic chemicals begin to leach from the leaves immediately1). The second stage of leaf decomposition is microbial colonization and growth, which in turn promotes leaf fragmentation due to invertebrate activity. Initial leaching from leaves is part of leaf decomposition. Since the conditioning of leaves begins in the terrestrial environment before defoliation, it is possible that leaching and conditioning of leaves occur simultaneously when the leaves enter the stream, possibly increasing after submergence, and becoming more palatable for macroinvertebrates. In stream water, the fragmentation of leaves resulting from feeding and case-building by insect larvae promotes leaching from the leaf materials.

In the present study, leaching from leaves generally increased over the 30-day study period. It is possible that the amount of materials that were leached exceeded the amount of material that could be consumed by the larvae and that the leachates from leaves are of relatively higher quality compared to nutrient derived from the leaves themselves. Indeed, the inverse relationship observed between shredder size and the rate of DOC production from shredded leaf litter may be a reason for the increases of TC concentrations during the experiment period. For example, DOC production may increase due to feeding by small individuals which likely shred the leaf material into finer pieces11). The pre-terminal and terminal larval instars used in this study were large, which may account for TC release rates being relatively low. Until the cuticle gradually disappears due to conditioning, the cuticle of *Quercus glauca* leaves may inhibit invertebrate feeding. Leaf toughness is considered to be an important factor affecting the feeding of larvae.

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. In addition to the physicochemical parameters measured in the present study, other dissolved materials would also be present in the leachate. Indeed, compared to leaf tissues which have a low assimilation efficiency11), the relatively higher quality and quantity of nutrients in the leachates may increase the survival of *Lepidostoma japonicum* larvae. This hypothesis may be supported by the experiment result that the mean EC values were lower in the experimental chambers than in the control chambers, except for chambers containing fewer than 12 leaves, indicating that some conductive materials were consumed by the larvae in these chambers.

5. CONCLUSION

In the present study, physicochemical parameters, such as EC, pH, TC, TN and concentrations of the major ions, were observed to increase in leachate produced by immersing leaves of the evergreen tree *Quercus glauca* in stream water samples with and without *Lepidostoma japonicum* larvae. The precise consumption rates of leachate from *Quercus glauca* by *Lepidostoma japonicum* larvae could not be accurately clarified as the number of larvae added to the experimental chambers containing leave samples was insufficient. However, the possibility that leachates from the *Quercus glauca* leaves are utilized by pre-terminal and/or terminal larval instars of *Lepidostoma japonicum* for growth was demonstrated. Furthermore, it was also demonstrated that leachates derived from the shredding activities of insect larvae affect the physicochemical characteristics of stream water. The role of shredders in generating FPOM particles through egestion and production of leaf fragments for feeding and case construction and how this affects stream water quality composition is widely known. However, the importance of shredders in generating dissolved organic compounds in stream has not been extensively investigated.

Since only leaves of *Quercus glauca* and larvae of *Lepidostoma japonicum* were used in the present study, the potential compounding effects of other leaf species or invertebrates were excluded. As a result, potential problems associated with predation, competition with other shredders, and natural food supply could be excluded in the present experiments. In addition, by enclosing the experimental system within chamber, the potential effects of current variation on leaf decomposition were also decreased. However, since the effect of conditioning duration, microbial colonization and growth would differ among leaves from different tree species, and because the preference for *Quercus glauca* leaves may vary among species, field experiments considering different leaf and insect species are considered necessary.

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アラカシ(*Quercus glauca*)リーフリターへのコカクツツトビケラ（*Lepidostoma japonicum*）による摂食・営巣行為が渓流水質に及ぼす影響と溶出成分利用の可能性について

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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，その溶出成分は渓流水質の形成に重要な役割を果たすものと思われる．河畔から渓流水中に落下したリーフリターは，その後の菌類・微生物増殖（conditioning）

や水生昆虫の摂食等を経て分解が進む．筆者は，これまで南九州河畔域に広く分布する常緑広葉樹であるアラカシリーフリターの溶出・分解特性の解明を進めてきたが，これに対する水生昆虫の影響については不明であった．そこで本研究は，南九州渓流に広く生息するコカクツツトビケラの摂食・営巣行為がアラカシリーフリターの溶出・分解特性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．さらには，コカクツツトビケラによる溶出成分の利用の可能性についても考察した．

2013年3月，一ツ瀬川支流竹尾川下流（宮崎県西都市）の河畔域に生育するアラカシの２年葉（落葉直前のもの）を採取し，十分な洗浄・乾燥と重量測定の後，1,000（ml）の現地渓流水を満たした5個の水槽（40×15×20cm）に1，3，6，12，24枚ずつ投入した．各水槽は各5個ずつ２組（合計50個）準備し，１組は調整区（control）とし，他の１組には2013年3月に同調査地で採取したコカクツツトビケラ幼虫を，筒巣サイズを計測後，6匹ずつ投入し，実験区（experimental）とした．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き，30日後，アラカシリーフリターの乾燥重量を計測し，コカクツツトビケラの成長率，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，陰イオン濃度を測定した．

30日間でのアラカシリーフリターの重量損失は，溶出のみで14.95～27.46%，コカクツツトビケラ幼虫の摂食・営巣行為によっては23～50%程度と推測された．また，成分の溶出に伴う水質変化が認められ，EC，pH，TC，TN，Na^+，NH_4^+，K^+，Cl^-・NO_3^- 濃度においては，調整区と実験区との間に有意な違いが認められた（$P<0.05$）．この違いの原因は，おもにコカクツツトビケラ幼虫の摂食・営巣行為に伴うアラカシリーフリターの破砕および微生物の増殖，ならびに幼虫が排出した糞によるものと推察された．さらに，溶出成分濃度が高い実験区において，コカクツツトビケラ幼虫の成長率と羽化率が高く死亡率が低いことが認められた（$P<0.005$）．

これらの実験結果から，コカクツツトビケラ幼虫は摂食・営巣行為を通じて，渓流水質の成分組成に影響を及ぼしているとともに，より質の高い栄養源として溶出成分を利用している可能性のあることが示唆された．