Research Notes

Role of *Anisocentropus* larvae in decomposition and leaching of riparian zone leaves

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Received October 1, 2015; Accepted February 1, 2016

From our previous studies, it is necessary to examine multiple aspects of the natural system simultaneously to better mimic natural conditions. In this note, it was, therefore, evaluated that the leaching characteristics of leaves of several of the dominant plant species in the riparian zone of the Takeo River in Miyazaki Prefecture, Japan and examine the possible utilization of leachates from these leaves 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.

To replicate natural stream conditions, as found in the study site, litter packs consisting of leaves from the dominant plant species of *Quercus glauca, Meliosma rigida and Styrax japonica* leaves were placed into chambers filled with stream water with *Anisocentropus* larvae. Furthermore, in order to clarify the weight loss and leaching rates of leaves in the absence of larvae, *Q. glauca, M. rigida*, and *S. japonica* leaves were placed separately into each of three beakers filled with stream water, respectively. After 30 days of incubation, all of the remaining leaf materials and the larvae were removed from each chamber and the weight loss of leaves, the rates of growth and pupation of *Anisocentropus* larvae, and cation concentrations of water were measured.

The order of weight loss rate among species was *S. japonica*＞*M. rigida*＞*Q. glauca* for every chamber. The growth of larvae cases was higher in the chambers that contained more leaves initially. NH_4^+, K^+, and Mg^{2+} were present in the chamber water at higher concentrations than in the stream water (initial concentrations) due to leaching from leaves and biological activity of larvae. K^+ was leached most abundantly in all samples.

In the present study, it was demonstrated that leaching rate, weight loss rate, and the amount of cations released differ among three species that are dominant in the riparian zone and predominate the leaf litter along the investigated stream. *Anisocentropus* larvae in the stream selectively utilize leaves of different species according to purpose, such as a food source and for case building. In turn, the pupation and emergence rates of larvae are affected by the leachate from leaves of these species.

Key words: leaching, leaf decomposition, riparian leaves, shredder, stream water composition.

1. Introduction

Allochthonous material, such as leaves, is the main energy source for consumers in small streams. 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 bacteria4) 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 invertebrates9) , thus facilitating the fragmentation process. Allochthonous organic material, such as leaf litter, is the source of 50% to 90% of the energy utilized by stream organisms8) .

This allochthonous organic matter is decomposed through a combination of physical, chemical, and biological processes1) , releasing inorganic nutrients3) and dissolved organic matter (DOM) 22) into the water. Since considerable quantities of allochthonous material enter lotic ecosystems from riparian forests6, 7) , substances derived from the decomposition of leaf litter can be expected to have a major impact on stream water quality.

Among the functional feeding groups in stream invertebrates4) , shredders accelerate the biological conversion and fragmentation of leaf litter (CPOM) to fine particulate organic matter (FPOM) and ultrafine particulate organic matter (UPOM) 1, 26) . 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 process16,17) , but the potential importance of shredders in generating dissolved organic matter in streams and the subsequent influence of leaf fragmentation on stream water composition has not been fully investigated.

Our previous experimental results [18,20], however, have indicated the possibility that pre-terminal and/or terminal larval instars of *Lepidostoma japonicum* and *Anisocentropus*,which are classified as shredders, utilize leachates (DOM) from *Quercus glauca* as well as CPOM to support growth in stream food webs and that leachates are derived from the shredding activities of insect larvae in addition to feeding and case building activities. However, since only leaves of *Q. glauca* were used, the potential compounding effects of other leaf species were excluded. To better mimic natural conditions, studies that examine multiple aspects of the natural system simultaneously are necessary.

In this note, we evaluate the leaching characteristics of leaves of several of the dominant plant species in the riparian zone of the Takeo River in Miyazaki Prefecture, Japan and examine the possible utilization of leachates from these leaves 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

Leaves used for the experiments were collected from the riparian zone of the Takeo River in Saito City, Miyazaki Prefecture. The Takeo River, a tributary of the Hitotsuse River, originates in the Southern Kyushu Mountains and flows from an elevation of 600 m to 100 m (Fig. 1). The geomorphology and vegetation of this reach of the stream were characterized in our previous study [18]. 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 1/40, and a mean current velocity ranging from 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 *Q. glauca, Symplocos theophrastifolia, Machilus japonica*, and *Litsea acuminata* Kurata and deciduous trees such as *Meliosma rigida* and *Styrax japonica*. 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.

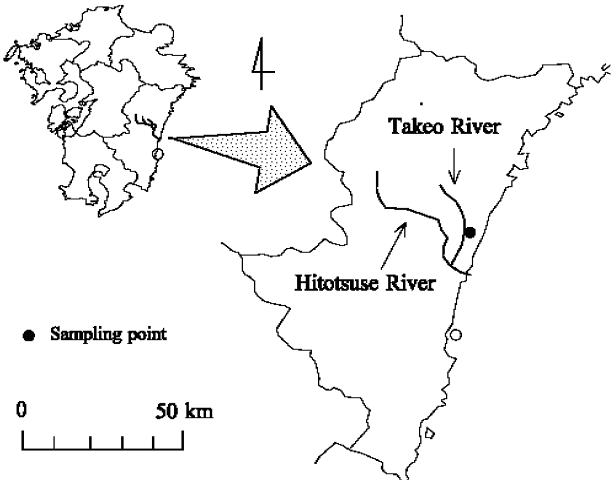

Fig. 1. Investigated site.

The invertebrate fauna of the stream 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 stream [13]. Larvae are most commonly found among deposits of allochthonous organic material in lentic areas such as pools and side pools in the stream.

Anisocentropus larvae are most common in the spring in side pools at the study site. Larvae are typically found associated with allochthonous organic material deposited on course to fine sand in slowly flowing water. *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 [23]. 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 [12].

Anisocentropus larvae are typically found in the lentic zones of streams, such as in side pools, where many species of leaves that are supplied from the terrestrial riparian zone accumulate.

2-2. Materials

Leaves from the dominant plant species and larvae from the dominant leaf-shredding macroinvertebrate species were collected from along a 50 m reach of the Takao River.

Leaves were collected from a tree of each species growing in the riparian zone of an exposed, unfertilized section of stream flowing over an alluvial plain. Leaves were collected from the dominant species as follows: 500 *Q. glauca* leaves, 200 *M. rigida* leaves, and 250 *S. japonica* leaves. 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. Whole leaves retained their shape without any skeletonization. Furthermore, a total of 100 *Anisocentropus* larvae were collected from side pools of the reach. All leaves and larvae were collected on March 23, 2015. Stream water (100 L) was collected from the same area of the river for use in laboratory incubation experiments.

2-3. Leaf processing in litter pack

2-3-1. Loss of leaf mass and growth of *Anisocentropus* larvae in 30-day incubation

In order to replicate natural stream conditions, as found in the study site, litter packs consisting of leaves from the dominant plant species in the study area were used in incubation experiments.

Collected leaves were washed with distilled water to remove aerosol dust and atmospheric gases, air-dried for 1 week, and then oven-dried at 80°C for 12 hr to eliminate water in the leaves. Four chambers (40 cm × 15 cm × 20 cm) were filled with 2000 ml of stream water, and *Q. glauca, M. rigida* and *S. japonica* leaves were randomly selected, weighed, and placed into each of the chambers in the following numbers to make two types of leaf litter packs: chambers A1 and A2 had 20, 15, and 50 leaves of each species, respectively, and chambers B1 and B2 had 30, 30, and 80 leaves of each species, respectively.

Then, 20 *Anisocentropus* larvae were randomly selected, blotted dry with a paper towel, and the wet weight and wet length of each larvae case were recorded before the larvae were placed in the chambers. The water in each chamber was aerated to simulate the physical environment of side pools of the river being investigated.

After 30 days of incubation, all of the remaining leaf materials and the larvae were removed from each chamber. The processed leaves and leaf fragments were collected using a sieve. The fragments were assayed for CPOM and FPOM. The leaves and fragments from each chamber were air-dried for 1 week, oven-dried at 80°C for 12 h, and weighed. Water was blotted from the surface of the larvae cases, and the wet length and wet weight of each were recorded.

2-3-2. Observation of evidence of feeding and case building behaviors on leaves

Anisocentropus larvae use leaves for food and leaf-disks for case building. Larvae graze and feed on the cuticle, epidermal, and mesophyll tissues, and they selectively undertake more extensive maceration of leaf tissue to make leaf-disc cases from a pair of ellipsoid leaf materials. Evidence of feeding and case building behaviors of *Anisocentropus* larvae remain on the leaves and can be easily distinguished. In the present experiments, the number of traces of feeding and case building behaviors was recorded for each leaf retrieved from the chambers after the 30-day incubation.

2-3-3. Measurements of cation concentration of water after 30-day incubation

Each leachate sample obtained in each chamber was filtered through a 0.20 μm filter immediately preceding determination of the concentration of cations (Li^+, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) in the water by ion chromatography (DX-120, Nippon Dionex K.K., Japan).

2-4. Evaluation of weight loss and leaching rate for leaves from each plant species under the larvae-free condition

In order to clarify the weight loss and leaching rates of leaves in the absence of larvae, 5 *Q. glauca*, 5 *M. rigida*, and 5 *S. japonica* leaves prepared as for the incubation with larvae tests were randomly selected, weighed, and placed into each of three beakers filled with 1000 ml of stream water, respectively. Each species had five replicates (sub-samples; a1~a5, b1~b5 and c1~c5), and the water in each beaker was aerated to simulate the physical environment of the side pools of the stream being investigated.

After 30 days of incubation, all pieces were removed from the beakers, air-dried for 1 week, oven-dried at 80°C for 12 h, and weighed. Each leachate samples were filtered through 0.20 μm filters immediately preceding determination of the concentration of cations in each beaker by the method described above.

3. Results

The overall comparisons of weight loss rates of leaves and feeding and case building rates by *Anisocentropus* larvae among chambers by plant species during the experimental period are shown in Fig.2. And the overall comparisons of weight loss rates of leaves and growth, pupation and emergent rates of larvae among chambers after incubation for 30 days are indicated as Fig.3.

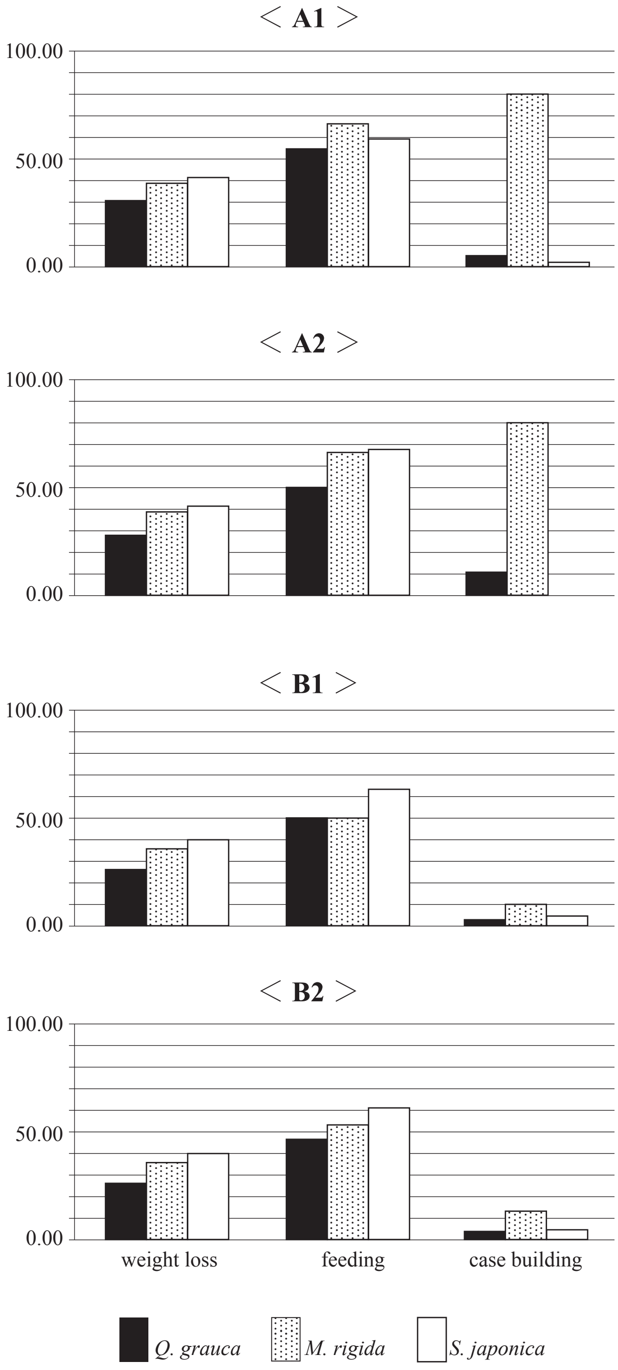

Fig. 2. Overall comparisons of weight loss rates of leaves (%) and feeding and case building rates by larvae (%) among chambers by plant species.

3-1. Leaf litter pack with larvae

3-1-1. Weight loss of leaves after incubation

Loss in weight and the number of leaves of each species in the litter pack in each chamber are reported in Table 1. Percent leaf weight loss was larger for chambers A1 and A2, which had a smaller initial weight of leaves than in chambers B1 and B2. The order of weight loss rate among species was *S. japonica*>*M. rigida*>*Q. glauca* for every chamber, indicating that the weight loss for *S.*

japonica leaves is nearly 1.5 times higher than that for *Q. glauca* leaves.

In these experiments, fragments (CPOM and FPOM) of leaves were produced from the egestion of particles and mechanical fragmentation of leaves during grazing and case building by invertebrates in each chamber. In the present experiment, however, the small number of *Anisocentropus* larvae placed in each chamber made only a small contribution to fragmentation (Table 2).

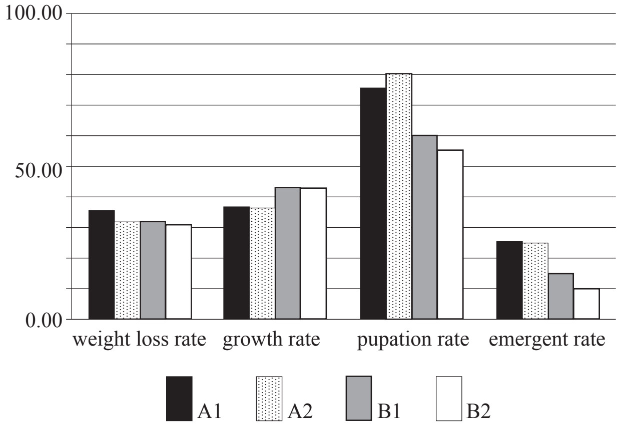

Fig. 3. Overall comparisons of weight loss rates of leaves (%) and growth, pupation and emergent rates of larvae (%) among chambers after incubation for 30 days.

Table 3 shows the utilization of leaves for feeding and case building by *Anisocentropus* larvae in each chamber by plant species. *S. japonica* showed the highest and *Q. glauca* showed the lowest utilization for feeding rate, except for in chamber A1, where there was no marked difference between *Q. glauca* and *M. rigida*. The rate of leaves being utilized for case building was highest for *M. rigida* in every chamber, but was especially high (80% of leaves) in chambers A1 and A2. On the other hand, *S. japonica* leaves were barely used for case building, especially in chambers A1 and A2, however, there is a lower number for *Q. glauca* in chamber B1.

3-1-2. Growth, pupation, and emergence rate of *Anisocentropus* larvae

Anisocentropus larvae used *Q. glauca* leaves for food, leaf-disc case building, and shelter in the experiments. All larvae survived the incubation experiments, and no dead larvae were observed.

The growth rate, pupation rate, and emergence rate of *Anisocentropus* larvae during the experimental period are shown in Table 4. Growth rate was determined as the increase in the total wet weight of larvae in each chamber. Insects that had emerged during the incubation were ex-

Table 1. Weight loss of leaves due to leaching and processing by larvae in 30-day incubation

Chamber	Species	Initial		After 30 days of incubation		Weight loss	
		Number of leaves	Weight（g）	Number of leaves	Weight（g）	（g）	（%）
A1	*Q. glauca*	20	4.72	20	3.28	1.45	30.72
	M. rigida	15	5.14	15	3.14	2.00	38.91
	S. japonica	50	2.71	46	1.58	1.13	41.70
A2	*Q. glauca*	20	4.78	20	3.47	1.31	27.41
	M. rigida	15	4.31	15	2.66	1.65	38.28
	S. japonica	50	2.97	48	1.74	1.23	41.41
B1	*Q. glauca*	30	8.72	30	6.44	2.28	26.15
	M. rigida	30	7.80	30	4.90	2.90	37.18
	S. japonica	80	5.05	76	3.05	2.00	39.60
B2	*Q. glauca*	30	7.95	30	5.89	2.06	25.92
	M. rigida	30	7.96	30	5.05	2.91	36.58
	S. japonica	80	5.13	72	3.07	2.06	40.25

Table 2. Comparison of weight loss among chambers normalized to initial weight of leaves

Chamber	Weight（g）				Weight loss	
	Initial	After 30 days of incubation				
	Leaves	Leaves	Fragments	Total	（g）	（%）
	①	②	③	④＝②＋③	⑤＝①－④	（⑤/①）×100
A1	12.57	7.99	0.18	8.17	4.41	35.08
A2	12.06	7.87	0.33	8.20	3.86	32.01
B1	21.57	14.39	0.28	14.67	6.90	31.99
B2	21.04	14.01	0.53	14.53	6.51	30.94

Table 3. Number and distribution of recognizable leaves utilized for feeding and case building by larvae

Chamber	Species	Initial		After 30-day incubation			
		Number of leaves	Weight (g)	Number of leaves		Utilization (%)	
				Feeding	Case building	Feeding	Case building
A1	*Q. glauca*	20	4.72	11	1	55.0	5.0
	M. rigida	15	5.14	10	12	66.7	80.0
	S. japonica	50	2.71	30	1	60.0	2.0
A2	*Q. glauca*	20	4.78	10	2	50.0	10.0
	M. rigida	15	4.31	10	12	66.7	80.0
	S. japonica	50	2.97	34	0	68.0	0.0
B1	*Q. glauca*	30	8.72	15	1	50.0	3.3
	M. rigida	30	7.80	15	3	50.0	10.0
	S. japonica	80	5.05	51	4	63.8	5.0
B2	*Q. glauca*	30	7.95	14	1	46.7	3.3
	M. rigida	30	7.96	16	4	53.3	13.3
	S. japonica	80	5.13	49	3	61.3	3.8

Table 4. Growth, pupation and emergence rates of *Anisocentropus* larvae after incubation for 30 days

Chamber	Larvae case weight (g)		Weight gain	Growth rate	Pupation rate	Emergence rate	Pupation and emergence rate
	Initial	Final	(g)	(%)	(%)	(%)	(%)
	①	②	③＝②－①	(③/①)*100	④	⑤	④＋⑤
A1	1.52	2.07	0.55	36.33	75.00	25.00	100.00
A2	1.58	2.15	0.58	36.51	80.00	20.00	100.00
B1	1.47	2.10	0.63	43.00	60.00	15.00	75.00
B2	1.62	2.32	0.70	42.90	55.00	10.00	65.00

cluded from the data shown in Table 4.

The growth of larvae cases (weight gain) after the incubation was 0.55 and 0.58 g in chambers A1 and A2, respectively, and 0.63 to 0.70 g in chambers B1 and B2, respectively, indicating that weight gain was higher in the chambers that contained more leaves initially. While this trend was observed for growth rate, pupation and emergence rates were higher in the chambers with fewer leaves (chambers A1 and A2). Given the possibility of some leaf cases being ingested by either the individual or another *Anisocentropus* larvae after building a new case based on finding a few old cases in each chamber compared to the quantity of leaves used for case building as shown in Table 4, considerable damage to cases was expected in the chambers. However, no naked larvae were observed in any of chambers.

3-1-3. Changes in cation concentrations in solution

The concentrations of cations in each chamber after the 30-day incubation are shown in Table 5 along with a reference sample for stream water. The cations Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+} were detected in all of the samples tested; Li^+ was not detected in all samples and is not reported.

NH_4^+, K^+ and Mg^{2+} were present in the chamber water at higher concentrations than in the stream water (initial concentrations) due to leaching from leaves and due to the biological activity of larvae. Na^+ and Ca^{2+} were present at concentrations that were about the same or smaller than the initial concentration (stream water), indicating that these cations were consumed during the incubation. Of the cations, K^+ was leached most abundantly by all samples in the present study.

Table 5. Cation concentrations in water after 30 days of incubation

Chamber	Cation concentrations (mg/l)				
	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
A1	6.353	2.066	33.624	3.950	5.023
A2	3.325	1.615	16.788	2.034	2.584
B1	4.909	1.902	30.240	3.429	4.096
B2	4.125	1.678	29.326	2.645	3.152
Stream water	4.651	0.836	0.763	1.979	5.895

3-2. Species-specific weight loss and cation release for individual leaves in the absence of larvae

Table 6 shows the weight loss of individual leaves and changes in cation concentrations in culture water (per gram of leaf mass) for 30-day incubation in the absence of larvae. Under the larvae-free condition, the main cause of weight loss in leaves is leaching. In decreasing order, the weight loss (in grams) corresponding with plant species is *M. rigida*＞*S. japonica*＞*Q. glauca*, the percent weight loss was about the same for *Q. glauca* and *M. rigida*. The percent weight loss by leaching range from 21.60 to 25.69, 22.61 to 35.07, and 32.11 to 40.21 for *Q. glauca, M. rigida*, and *S. japonica*, respectively.

For all plant species, the greatest release of cations in 30 days was for K^+, indicating the same tendency of cation release as for incubation experiments with litter pack and larvae. The general order of decreasing cation concentration for Na^+, K^+ and Mg^{2+} after 30 days by species was *S. japonica*＞*M. rigida*＞*Q. glauca* and for $NH_4^+$was *Q. glauca*＞*S. japonica*＞*M. rigida*. Significant differences ($P < 0.05$) were observed among leaf species for weight loss (g) and percent weight loss rate and all substances by one-way analysis of variance.

4. Discussion

4-1. Leaf weight loss during leaf processing and utilization of dissolved leachates for growth by *Anisocentropus* larvae

When leaves fall into streams, soluble organic and inorganic compounds immediately begin to leach into the water, as part of decomposition. In the second stage of leaf decomposition, microbial colonization and growth promotes leaf fagmentation by invertebrate activity.

For deciduous leaves, most leaching occurs within the first few days of submergence, and from 25% to 85% of the weight of leaves is lost, depending on species, even in a larvae-free condition [11, 24, 27]. In contrast to quick decomposition for deciduous species, evergreen leaves of *Q. glauca* in a previous study lost less about 25% of their initial weight after 30 days [16]. In the present experiments, the weight loss determined for evergreen leaves of *Q. glauca* and deciduous leaves of *M. rigida* and *S. japonica* during initial leaching period in larval-free condition showed good correspondance to the ranges obtained in previous experiments. Overall weight loss from evergreen leaves of *Q. glauca* was less than that from deciduous leaves of *M. rigida* and *S. japonica* in the present experiments. These differences may be due to differences in the outer surfaces of the leaves. *Q. glauca* leaves have a tough cuticle on the surface, which causes these leaves to decompose more slowly than the leaves of other more quickly decomposing species. *M. rigida* leaves decomposed faster than *S. japonica* leaves. This is because, in part, *S. japonica* leaves are thinner than *M. rigida* leaves. *S. japonica* leaves lose nearly 40% of their weight solely through leaching during 30 days of submergence.

For assemblages of leaves in an incubation chamber for 30 days with invertebrate larvae, the overall net percentage processing of *Q. glauca* leaves varies with invertebrate species. *L. japonicum* larvae produced a decrease of approximately 23% to 50% depending on the amount of food supplied [19], while the decrease due to *Anisocentropus* larvae ranged from 16% to 27% [20]. In the present experiments, weight loss in *Q. glauca* leaves for 30 days by *Anisocentropus* larvae range from 25.92 to 30.72%, depending on the initial weight of the leaves with slightly higher weight loss rates than those in the previous experiment. Because approximately 8% to 10% of the weight loss is considered to be due to microbial processing [10], it is expected that weight loss attributable solely to larvae is slightly lower than the measured values. Weight loss of the three species in these experiments in decreasing order is *S. japonica*＞*M. rigida*＞*Q. glauca*, which is the same order as for the initial leaching rates.

The *Anisocentropus* larvae in this experiment fed on the epidermal and mesophyll tissues of *Q. glauca* and *M. rigida* leaves. In addition, larvae used these leaves for constructing cases. The sum effect of these biological

Table 6. Leaching based on weight loss of leaves and cation concentration by plant species

Beaker	Species	Weight of leaves		Weight loss		Cation concentrations in water（mg/l）				
		Initial	After			Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
		（g）	（g）	（g）	（%）					
a1	*Q. glauca*	1.01	0.75	0.26	25.69	1.130	5.698	2.798	2.528	3.936
a2	*Q. glauca*	1.05	0.79	0.26	25.00	1.912	6.591	4.168	3.587	5.488
a3	*Q. glauca*	0.83	0.65	0.18	21.60	0.120	0.443	0.207	0.135	0.200
a4	*Q. glauca*	0.96	0.72	0.24	25.00	1.230	5.724	0.854	2.634	4.714
a5	*Q. glauca*	0.98	0.77	0.21	21.52	0.800	1.390	1.706	1.718	0.952
b1	*M. rigida*	5.24	3.40	1.84	35.07	2.725	0.561	4.657	0.763	0.949
b2	*M. rigida*	3.29	2.52	0.77	23.48	5.224	0.630	10.027	2.036	2.856
b3	*M. rigida*	3.45	2.67	0.78	22.61	4.443	0.384	8.859	2.032	3.144
b4	*M. rigida*	2.93	2.25	0.68	23.04	4.911	0.514	9.284	2.459	3.871
b5	*M. rigida*	4.44	3.43	1.01	22.64	5.125	0.303	8.956	1.657	2.493
c1	*S. japonica*	0.93	0.61	0.32	33.87	6.310	1.031	10.290	3.277	8.931
c2	*S. japonica*	0.97	0.58	0.39	40.21	5.243	1.763	9.557	3.188	7.694
c3	*S. japonica*	0.76	0.51	0.25	32.24	6.833	1.485	10.274	3.982	12.541
c4	*S. japonica*	0.95	0.64	0.31	32.11	4.952	0.774	9.316	3.141	10.546
c5	*S. japonica*	0.81	0.53	0.28	34.57	5.714	1.911	10.684	3.741	12.281

leaf processing behaviors produced fragments (CPOM and FPOM in the present experiment) in previous studies [1, 18, 23]. However, few fragments were generated in each chamber in the present study, implying that either the generated COPM and FPOM was produced by larval egestion or that the amount of FPOM generated was too small to detect due to the small number of *Anisocentropus* larvae introduced into each chamber. There were no considerable differences in the number of leaves with feeding traces among three species in each chamber, but the number of *M. rigida* leaves with evidence of case building was remarkably high in chambers in which the number of leaves supplied was relatively small. *Anisocentropus* larvae, therefore, may show a preference for *M. rigida* leaves for case building under these circumstances. *Anisocentropus* larvae show very low utilization of *S. japonica* leaves for case building because the thin *S. japonica* leaves do not have sufficient stiffness to maintain the case.

Q. glauca leaves, which have a tough surface consisting of cutin, seem to be suitable for case building based on our previous study [20]; however, it decomposes more slowly than leaves of other more quickly decomposing species. Until the cuticle gradually disappears due to conditioning, the cuticle of *Q. glauca* leaves may inhibit invertebrate feeding. *M. rigida* leaves do not have a cuticle, but they also do have a tough surface. Leaf toughness is considered to be an important factor in determining the utilization of leaves for feeding and case building by larvae.

The weight loss for leaves in each experiment was broadly categorized as weight loss due to leaching and microbial 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. However, it is difficult to precisely distinguish the percent weight loss due to grazing and case building.

4-2. Growth, pupation, and emergence rates of *Anisocentropus* larvae

In the present study, larval growth rates were higher in chambers B1 and B2 for which the initial weight of leaves was greater; however, consumption of leaves was higher in chambers A1 and A2 for which the initial weight of leaves was lower. The amount of leachates released from leaves into the water increased with the leaf biomass submerged in the water [15, 17]. It is, therefore, possible that *Anisocentropus* larvae, which are shredder species, prefer high quality food [2] and utilize leachate as a high quality food resource instead of the leaves, which would afford a low assimilation efficiency, in chambers B1 and B2.

In a previous study with *Q. glauca* leaves and *Lepidostoma japonicum* larvae [19], rates of *L. japonicum* larval growth and emergence increased in chambers that contained large amounts of leaf material. In a previous experiment with *Q. glauca* leaves and *Anisocentropus* larvae [20], chambers with more leaves had higher larval growth and emergence rates and lower larval mortalities, as well as higher leachate concentrations. In the present experiment using leaves of *Q. glauca*, *M. rigida*, and *S. japonica* and *Anisocentropus* larvae, pupation and emergence were relatively lower in water that contained relatively more leaf material.

It is likely that the abundance of food facilitated the growth and subsequent emergence of *L. japonicum* larvae [19], and it is also possible that a high quality food supply controls the *Anisocentropus* life cycle through its impact on metabolism and is manifested in better feeding and growth rates such that growth and emergence of *Anisocentropus* larvae are encouraged. Alternatively, *Anisocentropus* larvae may have reallocated resources to preferentially pupate and emerge in order to escape from the conditions of the chamber in the experiment, as it deviated from those of the natural stream. Invertebrate growth rate and, to some extent, survivorship, are both affected by food quality as well as quantity [5]. Major soluble carbon compounds released from leaves are primarily carbohydrates and polyphenol compounds [20], which are indispensable for invertebrate growth. In the present experiments, pupation and emergence of *Anisocentropus* larvae might be affected by some leachates released from *M. rigida* leaves and *S. japonica* leaves.

4-3. Cation release characteristics of leaves after submergence for 30 days

In the pattern of leaching from leaves over time for each cation, deviation from the initial cation concentrations of stream water used in the treatments was attributed to cations leaching from leaves, decomposition of the leachates [27], and production and consumption by microorganisms and *Anisocentropus* larvae present in the treatments.

The most dominant cation was K^+, accounting for around 63% to 71% of all cations in each treatment. By day 30, NH_4^+, K^+ and Mg^{2+} concentrations were higher than the initial concentrations in all treatments. This observation suggests that these ions leached into the treatment water during leaf breakdown. The increase in NH_4^+ concentrations during the experimental period was corroborated by the findings from a previous study [25] that indicated that total nitrogen concentration in the leaves increases as decomposition proceeds because amino acids and proteins are synthesized by fungi that promote leaf decomposition.

In contrast to the high K^+ concentrations in the incubation water of leaching experiments, the initial K^+ concentrations in the stream water was lower than the other cations. The lower concentration of K^+ in the stream water suggests that there is a differential utilization of K^+ by the biota and that stream sediment acts as an important agent for the removal of leachates from water [21]. Clay in the sediment may bind and retain K^+. Potassium may also be retained by stream biota if there is a net increase in biomass. Most Na^+ found in stream water is derived from rock weathering, but some Na^+ may be supplied through rainwater. Closer to the ocean, as for the study site in this investigation, rainwater may contribute significantly to the ion supply [1].

In the insect-free leaching experiments, it was demonstrated that cations are leached from evergreen leaves, such as *Q. glauca*, when submerged in water for 30 days [14, 15, 16]. In the present study, however, the quantity of K^+ leached was one order of magnitude higher than the other cation concentrations in *M. rigida* and *S. japonica* leaves, showing much higher concentrations than in the previous experiments [19, 20]. The reason for this may be that *S. japonica* and *M. rigida* leaves, which were also used, released more K^+ than did *Q. glauca* leaves. It is also possible that using more than three times the number of *Anisocentropus* larvae per chamber than in the previous experiments accelerated biological decomposition through activities such as grazing and case building behaviors of invertebrates, which in turn accelerated the leaching processes of water-soluble substances [20].

Conclusion and Acknowledgments

In the present study, it was demonstrated that leaching rate, weight loss rate, and the amount of cations released differ among three species that are dominant in the riparian zone and predominate the leaf litter along the investigated stream—*Q. glauca*, S. japonica, and *M. rigida*. *Anisocentropus* larvae in the stream selectively utilize leaves of different species according to purpose, such as a food source and for case building. In turn, the pupation and emergence rates of larvae are affected by the leachate from leaves of these species.

Leaf processing rates, such as weight loss rate and cation release rate, however, fluctuate seasonally and are species-dependent. Leaf processing rates also depend on the age of the leaves and the extent of conditioning of the leaves[20]. Further statistical treatments of the data are necessary to show significant differences in the present results in full consideration of the conditioning[9, 14, 16] characteristics of each leaf species.

This study was supported by a research grant from Minami-Kyushu University

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