

Leaching characteristics of *Trapa japonica* in a non-eutrophic irrigation pond

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Trapa japonica is considered to be important biotic components of aquatic ecosystems because of its removal capacity of nutrients from the water. However, *Trapa japonica* is also considered to be a problem species in many of the lakes and ponds in which these environments have become increasingly eutrophic in response to the increase in organic runoff from urban and agricultural areas.

Most of the studies on the leaching and decomposition characteristics of *Trapa japonica* have been undertaken in eutrophic water bodies. Information in non-eutrophic (oligotrophic) condition, however, is extremely scarce. This paper therefore examined the leaching and subsequent decomposition characteristics of *Trapa japonica* under non-eutrophic conditions by laboratory leaching experiments and field measurements of an irrigation pond.

Trapa japonica releases a variety of substances into water bodies, including carbon, nitrogen, and ions. The concentrations of these substances in the pond examined in this study were observed to fluctuate seasonally, depending on water temperature, initial contents of materials, dissolved oxygen concentration (DO), flow rates into the pond, and consumption by aquatic flora and fauna.

The present results showed that the release rates of phosphorus and nitrogen from *Trapa japonica* were extremely low, and thus that the plant does not contribute to water quality degradation in the pond. Even under non-eutrophic conditions, however, parameters, such as TC (total carbon), TN (total nitrogen), Na^+ , Cl^- and SO_4^{2-} , tended to be absorbed by *Trapa japonica* and then released when the plants became submerged. The amounts of these accumulated substances that were released, was in proportion to the amounts of these compounds that were accumulated by *Trapa japonica*. It is therefore important to identify which substances are accumulated and then selectively released by *Trapa japonica* in order to manage the ponds and lakes colonized by this species.

Key words : *Trapa japonica*, leaching characteristics, non-eutrophic pond, water quality.

1. INTRODUCTION

Trapa japonica is an aquatic macrophyte that can become dominant in ponds, shallow lakes, and along river margins in Japan, particularly in shallow, nutrient-rich waters (0.3 to 2.0 m deep)²²⁾. The above-water parts of this annual herb consist of a floating rosette of leaves around a central stem, which makes it easy to distinguish from other floating aquatic plants. During the growing season, the older leaves and developing fruit move outwards, forming several layers of leaves as new leaves are formed²⁴⁾.

The mature fruit separate from the plant and sink to the bottom of the pond or lake where they overwinter and germinate during the warm season. Each plant can produce approximately 300 seeds a year²⁸⁾, implying that the species has a high fecundity.

Aquatic plants such as *Trapa japonica* are considered to be important biotic components of aquatic ecosystems, particularly because they are involved in the removal of nutrients from the water³⁾. Indeed, the ability of *Trapa japonica* to uptake nutrients rapidly has meant that several efforts have been made to use this species to reduce nutrient levels in polluted environments in Japan^{4,17,26,31)}.

However, in Japan, *Trapa japonica* is also considered to be a problem species in many of the lakes and ponds in which these environments have become increasingly eutrophic in response to the increase in organic runoff from urban and agricultural areas³¹⁾. Of particular concerns are the increases in nutrients, such as phosphorus and nitrogen, which are leached into the water from decaying *Trapa japonica*, as this exacerbates the eutrophic conditions further.

Since the release of phosphorus and nitrogen from decaying aquatic plants (e.g. water milfoil) is increased in water to which nitrogen and phosphorous had been added⁷⁾, these fluctuations of pond water quality are expected to be the

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response of *Trapa japonica* itself to excessive nutrients under eutrophic conditions when *Trapa japonica* grows out of control in a pond or lake in which there are excess nutrients, such as phosphorus and nitrogen in the water.

Most of the studies on the leaching and decomposition characteristics of *Trapa japonica* described above have been undertaken in eutrophic water bodies. Information in non-eutrophic (oligotrophic) condition, however, is extremely scarce. This paper therefore examined the leaching and subsequent decomposition characteristics of *Trapa japonica* under non-eutrophic conditions in order to increase our understanding of the effects of *Trapa japonica* decomposition on water quality.

2. SITE DESCRIPTION

2-1. Investigated pond

The study was conducted at an artificial irrigation pond called "Maeyama-ike" located in the southeastern Miyakonojyo basin in Mimata Town, Miyazaki Prefecture, Japan. The pond is located in the upper reaches of the Toshimi River, a tributary of the Oyodo River (Fig.1). In addition to the pond itself, the natural stream flowing into the pond ((a) in Fig.1) and the artificial channel flowing out of the pond were also studied ((b) in Fig.1). The pond is surrounded by an embankment and has a total area of 4,800 m² and a depth of 2 to 3 m. The southern bank of the pond is covered with riparian forest consisting of *Quercus glauca*. The other dominant aquatic plant species in the pond was *Egeria densa*. There was no waste water runoff into the pond and the turbidity was negligible.

2-2. Plant's growth in the pond

The growth characteristics of *Trapa japonica* in the pond are as follow. Seeds of *Trapa japonica* on the bottom of the pond near the inflow at a depth of approximately 2 m started to germinate in May and root in the

hydrosol. These plants produced new leaves from a central terminal meristem in a rosette pattern near the water surface. The inconspicuous flowers are borne in the leaf axils of younger leaves above the water during June to July in the pond. These floating leaves spread radially and cover the surface of the water body. At least 25 % of the water surface was covered with immersed and floating leaves by late August. After September, newly developed leaves were smaller than the existing leaves and remained below the water surface. Consequently, the immersed leaves then gradually moved toward the surface where they floated and then rapidly decreased entirely after December.

3. METHODS

In the present study, the effects of the materials leached from the *Trapa japonica* at the inflow of the pond on the water quality of the pond were measured.

3-1. Materials

Three *Trapa japonica* plants were collected from the pond. The portion of the plants that were actively growing were selected, on August 29, and October 20, 2011. Each *Trapa japonica* plant was sorted into leaves (floating leaves), stems, fruits and roots, and cut into approximately one-centimeter sections, except for the fruits.

3-2. Measurement of initial carbon and nitrogen contents

The plant materials collected on August 29 were then washed thoroughly with distilled water to remove dust, attached snails, algae, and any other matter, before air-drying for one week and then oven-drying at 80°C for 48 hours. Five one-centimeter plant sections were then selected and approximately 2 mg was removed from the center of the section. The initial carbon and nitrogen content of each 2 mg sample was then analyzed using an ash-

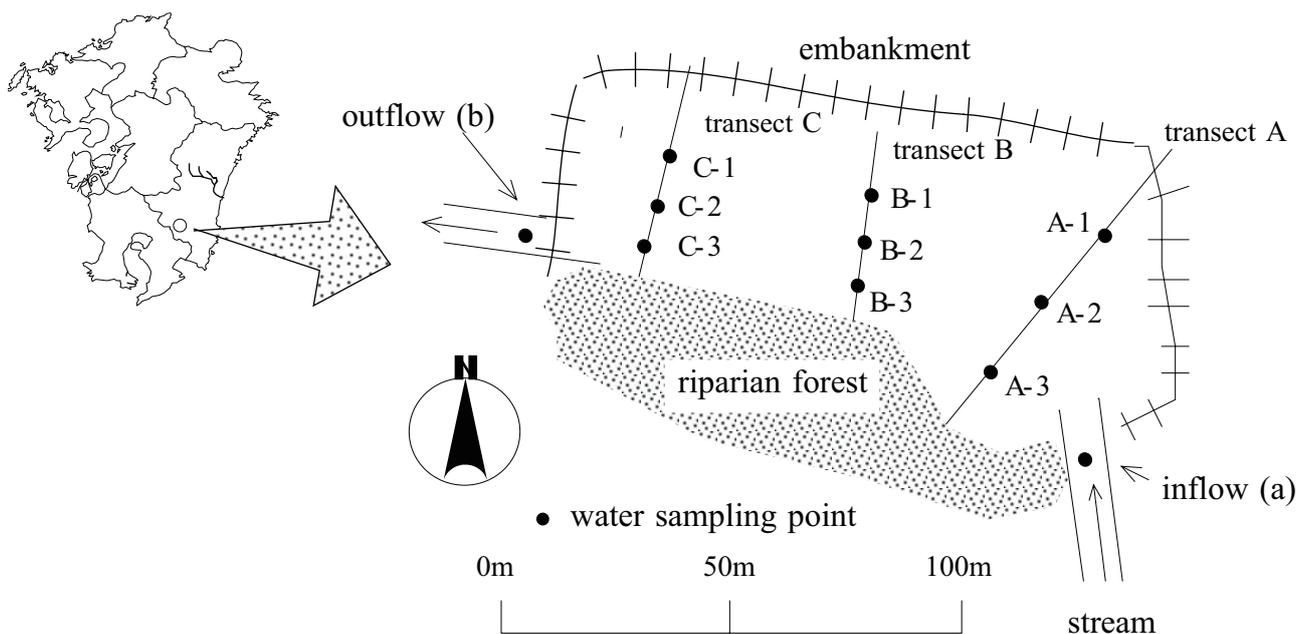


Fig. 1. Study pond and sampling points along transects A, B, C and in the inflow and outflow.

free CHNS/O Analyzer (2400 Series II, Perkin Elmer, Inc., America). The initial carbon and nitrogen contents of fruits could not be measured because of trouble of the analyzer.

3-3. Laboratory leaching experiments

The one-centimeter plant sections were dried as described above (3-2). About 1 g of each plant part was then randomly selected and weighed. Then, each sample was placed into a beaker filled with 500 cc of distilled water and allowed to stand for 30 days at ambient temperature (25-30°C). A total of five subsamples for each plant part were prepared.

On the 30th day of submergence, the plant materials of each subsample were dried as described above (3-2) and weighed. After each water subsample was filtered through a 0.20 µm filter, the electric conductivity (EC) and hydrogen ion concentration (pH) of the water samples were measured with a water quality probe (WQC-20A, TOA Electronics Ltd., Japan). The concentrations of 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), and the concentration of total carbon (TC; including total organic carbon (TOC) and inorganic carbon (IC)) and total nitrogen (TN) were measured using an automatic total organic carbon analyzer (TNC-6000, Toray Engineering K.K., Japan).

3-4. Sampling and analysis of pond water

Water samples for analysis were collected at the inflow and outflow of the pond and in the pond at two-month intervals on June 15, August 29, October 20 and December 22, 2011. Water samples were collected by hand using a 250 ml polyethylene bottle approximately 5 cm below the water surface. In the pond, water samples were also collected approximately 5 cm and 2 m below the water surface at approximately 40 m intervals along the right and left shores of the pond, as well as in the middle of the pond along the main current using a boat (transects A, B and C in Fig.1). A water sampler was used to collect water sample 2 m below the water surface.

After measuring the EC, pH and temperature of the water with a water quality probe (WQC-20A, TOA Electronics Ltd., Japan) *on site*, the collected water samples were transported to our laboratory. Water samples were filtered through a 0.20 µm filter, the concentrations of the cations, anions, TC and TN were measured. The measurements were conducted using the same methods applied to the stream water described above. In addition, dissolved oxygen (DO) of each sample was measured with a probe DR-700 (Hach Inc., America).

At the inflow ((a) in Fig.1), the width, depth and velocity of the channel were measured to estimate the flow at the time of sampling. The percentage of the water surface covered with submerged and floating leaves of *Trapa japonica* was recorded by visual inspection at each sampling time.

4. RESULTS

4-1. Inflow discharge and seasonal fluctuations in the area of floating leaves

The inflow discharge at the inflow which is simply calculated by multiplying the width by the depth by the velocity on each measurement time are 0.12, 0.13 0.01, and

0.005 (m^3/sec), in June, August, October and December, respectively.

Trapa japonica generally grows best in nutrient-rich waters²². Although the species can grow in waters up to 5 m deep, it prefers shallow waters (0.3 to 2.0 m). Since the pond in the study was 2 to 3 m deep, it was not actually well suited to growth of *Trapa japonica*.

The area covered with floating leaves (%) was assessed by visual inspection as being 0, 25, 14 and 0 % in June, August, October and December, respectively. In the study pond, *Trapa japonica* begins to emerge from the water surface after July. After August, smaller, newly developed leaves did not emerge from the surface. The area of floating leaves, therefore, reached the maximum in August, and then decreased by self-thinning until they disappeared completely in December. Of the various effects that *Trapa japonica* has on the pond, growth of the plant is considered to have a negative impact on the aesthetic value and water quality¹. The plant forms a dense mat of leaves and root systems that cover large areas of the surface, making it difficult to row a boat along transects A and B in August and October.

4-2. Initial carbon and nitrogen contents of parts of *Trapa japonica*

The mean initial carbon contents (\pm standard error) in the leaves, stems and roots were 39.5 ± 0.1 , 29.8 ± 1.2 and 26.6 ± 0.5 %, respectively. The mean initial nitrogen contents in the leaves, stems and roots were 3.2 ± 0.1 , 1.5 ± 0.1 and 1.3 ± 0.1 %, respectively.

Differences in carbon and nitrogen contents among plant parts were analyzed by one-way ANOVA (Table 1). Since the results for carbon and nitrogen contents among parts were significantly different ($P < 0.0001$), differences among individual parts were estimated using *Scheffe's* multiple range test (Fig.2). For initial carbon and nitrogen contents, significant differences were observed between the leaves and the other parts (stems and roots) in *Trapa japonica* ($P < 0.0001$).

4-3. Leaching characteristics of *Trapa japonica*

4-3-1. Decrease in plant weight after submergence of *Trapa japonica*

Subtracting the final dry weight of the different plant parts (leaves, stems, roots and fruits) after submergence from their initial dry weight was used to calculate the decrease in the weight of the plants due to plants decomposition in water. No fragmentation or invertebrate damage to part materials was observed in any samples. The decrease in plant weight for the months of August and

Table 1. One-way ANOVA between C and N contents, and plant parts and month of the study period

dependent variable	n	the mean square	F	P
〈initial contents〉				
carbon content	2	219.417	79.898	<0.0001
nitrogen content	2	5.227	273.028	<0.0001
〈weight loss rates〉				
parts	3	0.146	151.134	<0.0001
month	1	0.015	15.741	<0.0001

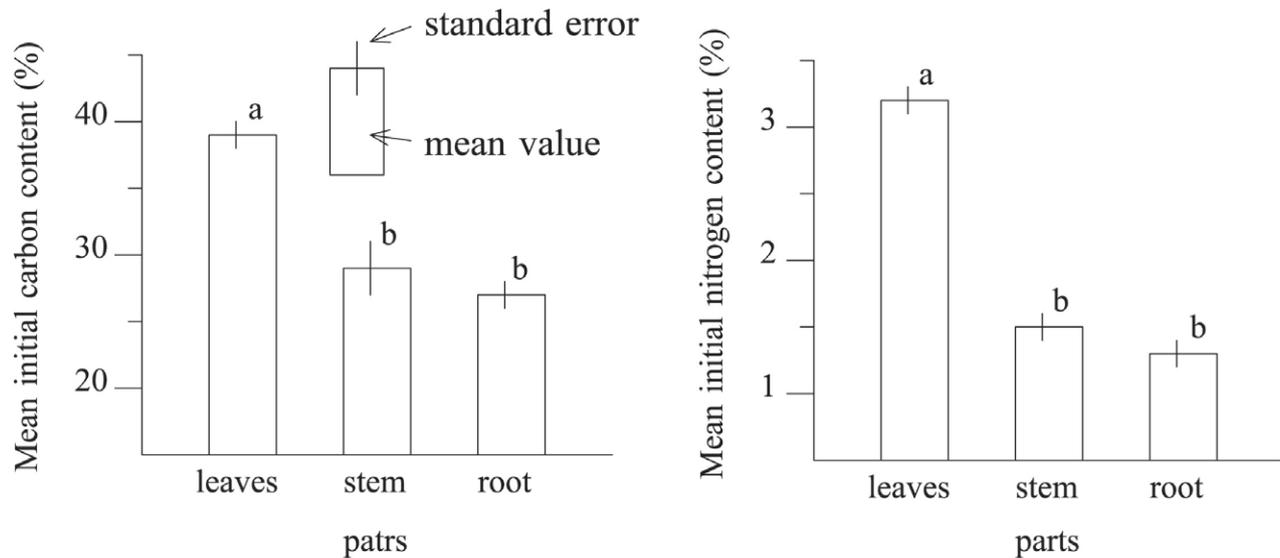


Fig. 2. Results of *Scheffe's* multipul range test. Letters above columns indicate statistically significant differences ($P < 0.0001$).

Table 2. The results of leaching experiment: Overall comparison of weight loss rates and physiochemical parameters of water corresponding to the experimental month in each measurement month

sampling date	parts	weight loss rate (%)	E.C. ($\mu\text{S}/\text{cm}$)	pH	TC (mg/l)	TN (mg/l)	mean \pm standard error
							C/N
29 August	leaves	28 \pm 1	113.5 \pm 2.8	6.7 \pm 0.1	89.6 \pm 1.5	3.1 \pm 0.3	29.8 \pm 1.4
	stems	33 \pm 1	342.1 \pm 7.2	6.7 \pm 0.1	36.5 \pm 2.2	1.4 \pm 0.1	25.3 \pm 2.4
	fruits	10 \pm 1	65.4 \pm 0.7	7.0 \pm 0.1	16.9 \pm 0.2	1.1 \pm 0.1	13.5 \pm 0.3
	roots	38 \pm 2	417.9 \pm 2.7	6.9 \pm 0.1	33.1 \pm 0.7	1.3 \pm 0.1	24.5 \pm 1.2
20 October	leaves	42 \pm 1	128.2 \pm 2.6	6.9 \pm 0.1	87.6 \pm 1.5	2.7 \pm 0.1	31.1 \pm 0.7
	stems	36 \pm 3	346.8 \pm 21.4	7.1 \pm 0.0	30.2 \pm 1.8	1.7 \pm 0.1	18.7 \pm 0.8
	fruits	11 \pm 2	81.6 \pm 4.5	7.3 \pm 0.0	14.4 \pm 1.1	1.9 \pm 0.2	7.4 \pm 1.9
	roots	34 \pm 1	263.1 \pm 6.4	7.0 \pm 0.1	31.2 \pm 0.8	1.7 \pm 0.1	16.5 \pm 0.3

October is shown in Table 2.

Differences in the weight loss rate among the different plant parts and the time of sampling were analyzed by one-way ANOVA (see Table 1). The results showed that there were significant differences in the weight loss rate of leaves and roots between August and October ($P < 0.0001$). Since the results for weight loss rate among the different plant parts at each time of sampling were significantly different ($P < 0.0001$), differences among individual parts were estimated using *Scheffe's* multiple range test. For the carbon and nitrogen contents, significant differences were observed between the fruit and the other parts of *Trapa japonica* ($P < 0.0001$) in each sampling date.

4-3-2. Changes in physiochemical parameters of water containing submerged *Trapa japonica*

1) Changes in EC and pH values of water containing submerged leaves

The pH and EC of distilled water samples containing submerged plant samples varied over time (Table 2). The initial EC and pH of distilled water was 1.36 $\mu\text{S}/\text{cm}$ and

6.9 $\mu\text{S}/\text{m}$, respectively. Marked increases in EC were observed after 30 days of leaf submersion in each sample in both measurement months, indicating that some materials leached from submerged plant parts.

The EC increased over time in all water samples, with significant differences observed after 30 days. In both months, the minimum EC values were observed in fruit. The lower EC values observed in water sample containing submerged fruit are likely due to the fact that the thick testa (outer seed cover) limits leaching.

The mean pH values of water in August decreased from, or were nearly equal to, the initial pH value after submersion in all water samples. However, the pH values in October increased in all water samples after 30 days of submergence. Unlike EC, no marked differences were observed in pH of water samples containing plants parts that had been submerged for 30 days.

2) TC and TN released from *Trapa japonica*

Mean TC and TN concentrations in the water samples containing plant parts (leaves, stems, roots and fruits) that

Table 3. The results of leaching experiment: Overall comparison of anions and cations concentrations of water corresponding to the experimental month in each measurement month(mg/l)

sampling date	parts	mean \pm standard error								
		Na ⁺	NH ₄ ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	NO ₃ ⁻	HPO ₄ ³⁻	SO ₄ ²⁻
29 August	leaves	5.11 \pm 0.10	1.59 \pm 0.02	11.82 \pm 0.05	2.12 \pm 0.01	2.68 \pm 0.39	9.54 \pm 0.16	0.18 \pm 0.05	0.40 \pm 0.20	0.01 \pm 0.01
	stems	7.00 \pm 0.25	0.67 \pm 0.20	25.00 \pm 0.92	1.56 \pm 0.16	0.98 \pm 0.09	30.63 \pm 2.69	0.08 \pm 0.05	0.64 \pm 0.24	1.07 \pm 0.37
	fruits	1.89 \pm 0.07	0.91 \pm 0.05	12.20 \pm 0.26	0.21 \pm 0.01	0.13 \pm 0.03	4.11 \pm 0.25	0.21 \pm 0.01	3.32 \pm 0.14	0.79 \pm 0.02
	roots	8.20 \pm 0.12	0.48 \pm 0.01	28.90 \pm 0.31	2.53 \pm 0.02	1.97 \pm 0.02	21.40 \pm 0.94	0.12 \pm 0.03	0.00 \pm 0.00	4.42 \pm 0.04
20 October	leaves	4.78 \pm 0.04	1.46 \pm 0.02	10.30 \pm 0.10	3.45 \pm 0.04	7.08 \pm 0.24	8.11 \pm 0.03	0.67 \pm 0.07	0.82 \pm 0.02	0.23 \pm 0.01
	stems	8.39 \pm 0.76	1.15 \pm 0.07	18.81 \pm 1.33	3.30 \pm 0.43	3.41 \pm 1.04	27.59 \pm 2.15	0.90 \pm 0.23	0.15 \pm 0.08	2.52 \pm 1.02
	fruits	1.38 \pm 0.07	1.43 \pm 0.10	13.94 \pm 1.29	0.52 \pm 0.06	0.24 \pm 0.02	4.05 \pm 0.35	0.55 \pm 0.07	4.18 \pm 0.12	1.63 \pm 0.05
	roots	7.25 \pm 0.08	0.82 \pm 0.01	15.17 \pm 0.10	2.60 \pm 0.21	1.84 \pm 0.13	13.25 \pm 0.33	0.26 \pm 0.03	0.00 \pm 0.00	5.62 \pm 0.15

had been submerged for 30 days are shown in Table 2. In the present measurements, TC concentration was equal to TOC concentration, because no IC (inorganic carbon) was detected in any of the samples.

In both measurement months, the highest and lowest TC concentrations were observed in the water sample containing submerged leaves and fruits, respectively. The TN concentrations in each measurement month were highest in the submerged leaves sample, while the TN concentrations of the leachate from the other plant parts in each sample month were almost the same. There was no significant difference in TC and TN concentrations of the different leachates between the measurement months.

3) Ion concentrations leached from *Trapa japonica*

Table 3 shows the cations and anions concentrations leached from the plant parts submerged in distilled water. The cations assayed were Na⁺, NH₄⁺, K⁺, Mg²⁺ and Ca²⁺, and the anions assayed were Cl⁻, NO₃⁻ and SO₄²⁻. HPO₄³⁻ was not leached from roots in both measurement months. The cations and anions appeared to leach from the plant parts immediately after submersion and the ion concentrations increased over time in all water samples¹¹⁾.

Among the cations, K⁺ was leached most abundantly by all plant parts, particularly from the stems and roots in the present study, which is similar to the release pattern observed for Na⁺. The K⁺ concentration relative to the total cation concentration after 30 days exceeded 70% in leachate from submerged stems, fruits and roots except for the leachate from leaves. The order of average cation release was K⁺ > Na⁺ > Mg²⁺ \approx Ca²⁺ > NH₄⁺.

Anion concentrations in leachate from submerged leaves on day 30 were in the same range as those of cations, with the exception of Cl⁻. Among the anions, high concentrations of Cl⁻ leached from all plant parts, particularly from stems and roots; this was similar to the release pattern observed in K⁺. NO₃⁻ and SO₄²⁻ leached more in October than in August from all plant parts. HPO₄³⁻ was not observed to leach from the roots in any of the measurement months.

4-4. Fluctuations of the water quality in the pond

The measured parameters and concentrations of each material in the pond water are shown in Table 4. Because the measured parameters and concentrations were nearly homogeneous throughout the water column and similar

between the water surface and from a depth of 2 m, the concentrations of parameters along transects A, B and C were expressed as means with a standard error.

4-4-1. Water temperature, pH, EC and DO

The mean water temperature in the pond was 1-4°C higher than that at the inflow (stream water), except in December. EC values were higher in the pond in October and December. The mean of pH of the pond was exactly the same as that at the inflow in October and December; however the mean pH in the pond was higher than that at the inflow in June and August. Generally, DO concentrations were higher in the stream water (inflow) than in the pond, except for June. DO values could not be measured in October due to problems with equipment.

4-4-2. TC and TN

TC concentrations in the pond tended to be higher at transects A and B than at transect C, which had high densities of *Trapa japonica*. In addition, TC concentrations were higher in the pond than in the stream except for August. TN concentrations in the pond (transects A, B and C) did not differ markedly from those of the stream water at the inflow. However, TC concentrations in the pond tended to be higher along transect A.

4-4-3. Fluctuations in anion and cation concentrations at two month intervals

The mean concentrations of the major ions at two-month intervals are shown in Table 5. In the field measurements, Li⁺, NO₂⁻, Br⁻ and PO₄³⁻ were not detected. NO₃⁻ was not observed in December, except along transect A. The abundant ions in the pond were Na⁺ (cation), and Cl⁻ and SO₄²⁻ (anions). The concentrations of K⁺, the most abundant cation in the laboratory leaching experiment, was considerably lower than those of the other ions.

The concentrations of Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cl⁻ tended to be higher in the pond (transects A, B and C) than in the stream water (inflow), implying that these ions were supplied in the pond. The concentrations of NH₄⁺ (except for June), NO₃⁻, and SO₄²⁻, however, were lower in the pond than in the inflow water, indicating that these ions were either consumed or disappeared in the pond.

Na⁺ is typically found in association with Cl⁻ (Fig.3). And NH₄⁺ and NO₃⁻ concentrations corresponding to DO concentrations were shown as Fig.4.

Table 4. Physiochemical parameters of the pond water in each measurement month

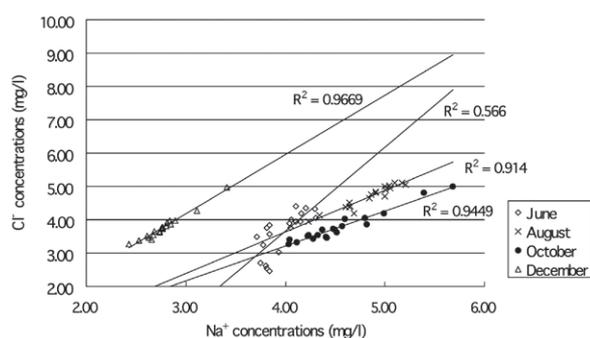
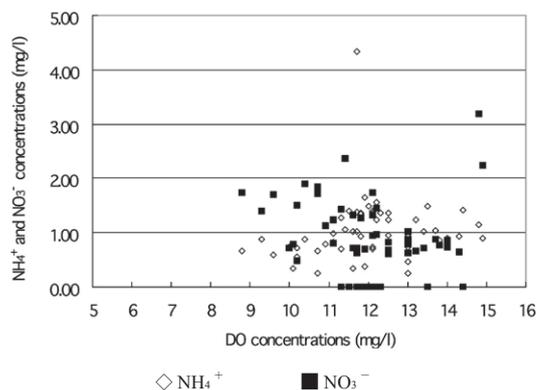
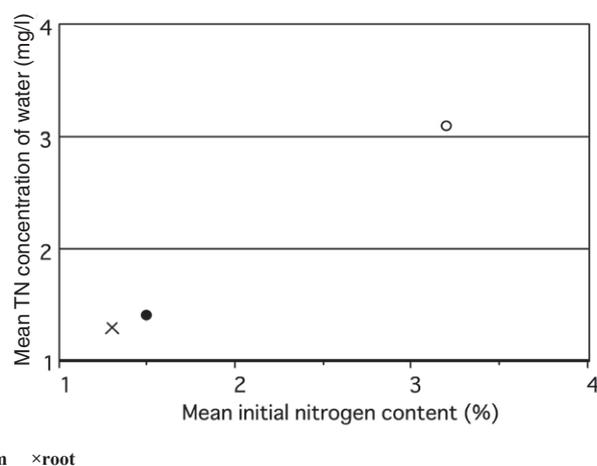
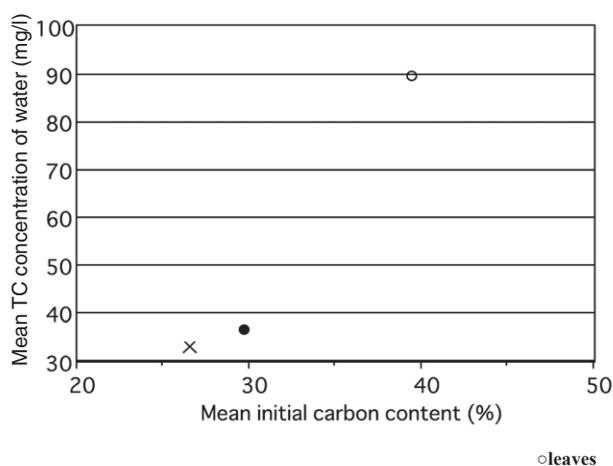
measurement month	sampling point	Temp. (°C)	E.C (μS/cm)	PH	DO (mg/l)	TC (mg/l)	TN (mg/l)	C/N	mean ± standard error
June	inflow	19.9	48.5	7.3	10.4	4.9	1.4	3.50	
	transect A	24.0 ± 0.9	50.8 ± 1.3	7.6 ± 0.1	12.6 ± 0.4	9.1 ± 0.3	2.1 ± 0.2	4.6 ± 0.4	
	transect B	23.5 ± 0.8	48.7 ± 0.3	7.6 ± 0.1	12.8 ± 0.7	5.9 ± 0.4	1.2 ± 0.1	4.8 ± 0.2	
	transect C	23.9 ± 0.8	48.0 ± 0.3	7.7 ± 0.2	12.3 ± 0.6	5.6 ± 0.1	1.3 ± 0.1	4.4 ± 0.3	
	outflow	24.2	49	7.5	10.7	5.6	1.2	4.67	
August	inflow	20.0	52.4	7.4	14.8	17.7	1.1	16.4	
	transect A	21.7 ± 0.4	55.9 ± 1.5	7.3 ± 0.1	11.5 ± 0.1	13.0 ± 0.2	0.9 ± 0.1	14.3 ± 0.8	
	transect B	22.0 ± 0.6	50.8 ± 1.0	8.1 ± 0.3	11.3 ± 0.8	14.0 ± 0.4	0.9 ± 0.1	16.5 ± 0.9	
	transect C	23.2 ± 1.0	51.1 ± 0.4	7.6 ± 0.2	10.4 ± 0.4	10.9 ± 0.2	1.0 ± 0.1	11.3 ± 0.7	
	outflow	24.6	49.9	8.4	11.6	11.4	0.9	12.1	
October	inflow	17.8	60.4	6.9	—	7.6	1.3	5.7	
	transect A	19.3 ± 0.2	62.8 ± 0.4	7.0 ± 0.1	—	9.2 ± 0.5	0.9 ± 0.1	11.1 ± 1.2	
	transect B	18.8 ± 0.1	61.9 ± 0.2	6.9 ± 0.1	—	8.6 ± 0.3	0.9 ± 0.1	9.6 ± 0.9	
	transect C	19.4 ± 0.2	61.6 ± 0.1	6.9 ± 0.1	—	9.0 ± 0.3	1.4 ± 0.1	6.9 ± 0.4	
	outflow	20.5	62.8	7.1	—	9.1	1.5	6.1	
December	inflow	11.6	58.9	7.0	13.0	11.2	1.3	9.0	
	transect A	10.4 ± 0.1	62.8 ± 0.3	7.0 ± 0.1	12.5 ± 0.2	11.4 ± 0.3	1.4 ± 0.1	8.2 ± 0.3	
	transect B	10.2 ± 0.1	62.4 ± 0.1	6.9 ± 0.1	11.9 ± 0.1	11.7 ± 0.3	1.3 ± 0.1	9.3 ± 0.4	
	transect C	10.0 ± 0.1	62.6 ± 0.1	6.9 ± 0.1	12.5 ± 0.5	11.3 ± 0.4	1.3 ± 0.1	8.8 ± 0.3	
	outflow	10.8	62.3	7.0	11.6	11.6	1.4	8.3	

Table 5. Ion concentrations of the pond water corresponding in each measurement month

measurement month	sampling point	Na ⁺ (mg/l)	NH ₄ ⁺ (mg/l)	K ⁺ (mg/l)	Mg ²⁺ (mg/l)	Ca ²⁺ (mg/l)	F ⁻ (mg/l)	Cl ⁻ (mg/l)	NO ₃ ⁻ (mg/l)	SO ₄ ²⁻ (mg/l)	mean ± standard error
June	inflow	3.77	0.24	0.55	1.17	2.47	0.05	3.25	0.95	4.43	
	transect A	4.15 ± 0.03	0.90 ± 0.05	0.85 ± 0.03	1.04 ± 0.03	2.46 ± 0.12	0.04 ± 0.01	4.07 ± 0.10	0.89 ± 0.03	4.54 ± 0.09	
	transect B	3.94 ± 0.07	0.90 ± 0.04	0.71 ± 0.04	1.01 ± 0.02	2.23 ± 0.07	0.03 ± 0.01	3.78 ± 0.24	0.75 ± 0.02	4.34 ± 0.03	
	transect C	3.82 ± 0.03	1.12 ± 0.65	0.62 ± 0.03	1.10 ± 0.02	2.41 ± 0.07	0.04 ± 0.01	2.95 ± 0.20	0.70 ± 0.03	4.25 ± 0.04	
	outflow	4.30	0.25	0.59	1.17	2.36	0.05	4.06	1.71	4.28	
August	inflow	4.34	1.15	0.85	0.56	1.07	0.02	4.14	3.18	3.33	
	transect A	5.05 ± 0.05	0.96 ± 0.03	0.98 ± 0.02	0.87 ± 0.02	1.76 ± 0.06	0.06 ± 0.01	4.99 ± 0.05	1.38 ± 0.23	4.25 ± 0.03	
	transect B	4.69 ± 0.11	0.81 ± 0.04	0.88 ± 0.02	0.77 ± 0.03	1.61 ± 0.11	0.04 ± 0.01	4.43 ± 0.14	1.48 ± 0.26	3.92 ± 0.13	
	transect C	4.81 ± 0.07	0.60 ± 0.05	0.86 ± 0.02	0.77 ± 0.01	1.52 ± 0.02	0.06 ± 0.01	4.74 ± 0.10	1.56 ± 0.08	1.93 ± 0.33	
	outflow	4.86	0.34	0.86	0.79	1.56	0.06	4.75	1.33	1.87	
October	inflow	4.23	1.46	0.82	0.83	1.73	0.00	3.54	3.46	4.60	
	transect A	4.81 ± 0.25	0.81 ± 0.07	1.20 ± 0.06	1.00 ± 0.01	2.05 ± 0.03	0.05 ± 0.01	4.05 ± 0.29	0.60 ± 0.06	3.84 ± 0.08	
	transect B	4.54 ± 0.11	0.93 ± 0.10	1.18 ± 0.02	0.97 ± 0.02	1.96 ± 0.03	0.05 ± 0.01	3.71 ± 0.12	0.56 ± 0.09	3.81 ± 0.08	
	transect C	4.38 ± 0.09	1.14 ± 0.10	1.11 ± 0.03	0.86 ± 0.02	1.75 ± 0.05	0.04 ± 0.01	3.67 ± 0.11	0.76 ± 0.21	3.74 ± 0.11	
	outflow	4.04	1.68	1.14	0.80	1.71	0.04	3.41	2.58	3.67	
December	inflow	2.77	1.90	0.87	0.88	1.90	0.03	3.72	3.04	5.11	
	transect A	2.73 ± 0.06	1.37 ± 0.05	1.11 ± 0.04	1.02 ± 0.01	2.34 ± 0.02	0.03 ± 0.01	3.71 ± 0.10	0.95 ± 0.15	4.81 ± 0.03	
	transect B	2.84 ± 0.11	1.35 ± 0.03	1.23 ± 0.07	1.05 ± 0.01	2.41 ± 0.03	0.03 ± 0.01	3.91 ± 0.22	0.00 ± 0.00	4.81 ± 0.07	
	transect C	2.73 ± 0.09	1.45 ± 0.04	1.18 ± 0.09	1.07 ± 0.03	2.41 ± 0.07	0.04 ± 0.01	3.66 ± 0.15	0.00 ± 0.00	4.86 ± 0.15	
	outflow	2.85	1.41	1.21	1.12	2.56	0.04	3.87	0.00	4.90	

Table 6. The results of multivariate analysis of variance between physicochemical parameters, sample site, time of sampling, and interaction

	month				line				month*line			
	n	mean square	F	P	n	mean square	F	P	n	mean square	F	P
temp.	3	668.282	338.737	< 0.001	2	1.680	0.852	n.s.	6	1.148	0.582	n.s.
EC	3	820.898	274.612	< 0.001	2	42.045	13.731	< 0.001	6	9.294	3.109	< 0.010
pH	3	3.345	35.194	< 0.001	2	0.305	3.210	< 0.050	6	0.317	3.337	< 0.010
DO	2	11.645	8.482	< 0.001	2	0.857	0.624	n.s.	4	1.176	0.857	n.s.
TC	3	118.775	184.393	< 0.001	2	13.097	20.332	< 0.001	6	8.101	12.576	< 0.001
TN	3	1.281	28.659	< 0.001	2	0.393	8.804	< 0.001	6	0.430	9.628	< 0.001
Na ⁺	3	15.509	230.250	< 0.001	2	0.427	6.338	< 0.005	6	0.099	1.481	n.s.
NH ₄ ⁺	3	1.134	4.912	< 0.005	2	0.054	0.235	n.s.	6	0.142	0.614	n.s.
K ⁺	3	0.846	72.152	< 0.001	2	0.058	4.927	< 0.010	6	0.032	2.743	< 0.050
Mg ²⁺	3	0.248	96.036	< 0.001	2	0.010	7.449	< 0.050	6	0.019	7.449	< 0.001
Ca ²⁺	3	2.378	96.216	< 0.001	2	0.139	5.640	< 0.010	6	0.082	3.307	< 0.010
F ⁻	3	0.021	12.423	< 0.001	2	0.003	1.840	n.s.	6	0.001	1.717	n.s.
Cl ⁻	3	4.208	25.758	< 0.001	2	1.169	7.155	< 0.005	6	0.523	3.202	< 0.010
NO ₃ ⁻	3	4.091	38.472	< 0.001	2	0.352	3.312	< 0.050	6	0.576	5.420	< 0.001
SO ₄ ²⁻	3	6.664	66.287	< 0.001	2	2.375	23.629	< 0.001	6	1.719	17.095	< 0.001

**Fig. 3.** Correlation between Cl⁻ and Na⁺ concentration of the pond water during the experimental period.**Fig. 4.** Correlation between NH₄⁺ and NO₃⁻ concentrations in the pond during the experimental period.**Fig. 5.** Overall comparison of the relation between mean initial carbon (nitrogen) content and TC (TN) concentration of water corresponding to the plant parts.

4-4-4. Statistical analysis

Table 6 shows the results of the multivariate analysis of variance. In this analysis, the measurement month (June, August, October and December) and sampling points (transects A, B and C) were regarded as independent variables and each measurement value as a dependent variable. Significant differences were observed in each of the measurement values depending on measurement month and sampling point. However, significant interactions were observed between each variable pair. The monthly distribution of TC, TN, Na⁺, K⁺, Cl⁻ and SO₄²⁻ concentrations, all of which were abundant in the laboratory experiments, along transects A, B, and C in the field are shown in table 4. The table shows a general tendency for these parameters to be higher along transects A and B.

5. DISCUSSION

5-1. Decrease in *Trapa japonica* plant mass after 30 days of submergence

In deciduous leaves, most leaching occurs within a few days of submergence. For example, autumn-shade leaves in water can lose up to 40% of their dry weight in a few days⁹⁾, and the ash-free dry weight of sugar maple, yellow birch, and beech was 85.4%¹⁸⁾ of the initial weight after 2 days of submergence. Up to 25% of the initial dry weight of some riparian deciduous tree leaves (e.g., *Alnus* sp., *Salix* sp.) are lost due to leaching in the first 24 h of submergence²⁹⁾. Among evergreen trees, leaves of *Quercus glauca* lost 4~7% after one day¹²⁾, which was similar to *Quercus alba* which lost 5.16% of its initial weight within 24 h²⁵⁾.

These experiments were conducted under lotic conditions in natural streams or in artificial channels, which have shorter retention times for leaves that enter the river system. However, the pond system investigated in the present study had longer retention times under lentic condition, which seems to affect the initial leaching characteristics of the plant organisms in the water body²³⁾. For example, maximum loss of soluble components due to abiotic leaching occurs 24 hours after exposure of leaf litter to water and is typically complete within approximately four weeks in a temperate woodland water body²⁷⁾. In the present experiments, the decrease in the mass of leaves, stems, fruits, and roots of *Trapa japonica* after 30 days of submergence was greater than that lost from evergreen *Quercus glauca* leaves after 30 days of submergence in water¹²⁾.

No significant relationship was observed between the decrease in plant mass and the concentrations of leached materials, except for EC, K⁺ and NO₃⁻, indicating that numerous substances may have leached from the different parts of the plant during decomposition (e.g. sugars, lignin, cellulose and hemicellulose)¹²⁾, and which would not have been detected by the instruments used in this experiment.

5-2. Effects of initial carbon and nitrogen contents on the leaching rates of TC and TN in *Trapa japonica*

The initial carbon and nitrogen contents values of the leaves, stems, and roots in *Trapa japonica* in August were relatively lower and higher, respectively, than those of the other plant species in the literature¹³⁾. However, among aquatic plants, the initial TC content of each different part of *Trapa japonica* is higher than that of water milfoil which was calculated to be 1.48%, while the initial TN content of each different part of *Trapa japonica* is lower

than that of water milfoil which was calculated to be 2.6%²¹⁾, except for the leaves.

The TC and TN concentrations leached from *Trapa japonica* in August were positively correlated to the initial carbon and nitrogen contents, respectively (Fig. 5). Thus, in *Trapa japonica*, the amounts and rates of carbon and nitrogen components leached from the different plant parts are influenced, in part, by the initial carbon and nitrogen contents of the plant. The TN concentrations were much lower than the TC concentrations in the pond. This is likely to have been because the N in the pond water, which is derived from decaying plant and animal material, would have been immediately used for further biomass production.

5-3. Ion concentrations

In the leaching experiments, the cations Na⁺, NH₄⁺, K⁺, Mg²⁺ and Ca²⁺ and the anions Cl⁻, NO₃⁻ and SO₄²⁻ were detected. HPO₄³⁻ did not leach from roots, while SO₄²⁻ leached from roots much more than from the other plant parts in both measurement months. The amounts of K⁺ leached from the plant parts were one order of magnitude higher than the levels of the other ions. The overall leaching characteristics of ions corroborated those of previous studies on evergreen species¹²⁾ and the aquatic macrophyte *Typha latifolia*, for which the release rates are K⁺ > Na⁺ > Ca²⁺ > Mg²⁺ > P²⁻. However, the release rates of Na⁺ and Cl⁻ were higher in *Trapa japonica* than they were in these other plants species. Thus, based on the results of the leaching experiments in the present study, it appears that the contribution of ions and leachate by *Trapa japonica* to the pond environment is substantial.

In contrast to the high K⁺ ratios in the leaching experiments, the K⁺ concentrations in the pond water were two orders of magnitude lower than those measured in the laboratory leaching tests, as well as being relatively lower than the concentrations of the other cations and anions. The low K⁺ concentrations observed in the pond in June and August may have been because K⁺ consumption is increased during plant and animal growth. In addition, this increase in plant growth and decrease in K⁺ availability correspond to seasonal changes in flow conditions in the pond. K⁺ appears to be completely water soluble in plants but may combine with organic compounds¹⁵⁾ and pond sediment acts as an important agent for removal of leachate from water¹⁴⁾. Therefore, the clay component of the sediment may serve as a reservoir of chemically bound K⁺.

The most abundant ions in the pond water were Na⁺, Cl⁻ and SO₄²⁻. As described previously, Na⁺ is typically found in association with Cl⁻ in the present study. Most of the Na⁺ present in natural water is derived from rock weathering, but some Na⁺ and Cl⁻ may be supplied through rainwater. Closer to the coast as the watershed of the investigated pond, rainwater may contribute significantly to ion supply. Judging from the relatively high Cl⁻ tolerance of *Typha latifolia*¹⁹⁾, the laboratory results of the present study suggest that, compared to the other ions, more Cl⁻ is released from *Trapa japonica*, especially from the stem and root tissues. Furthermore, these results imply that Cl⁻ may be taken up by *Trapa japonica* and released easily.

Large amounts of SO₄²⁻ accumulate in watersheds, and much of SO₄²⁻ is incorporated into plant biomass¹⁸⁾. Some of the sulfate is returned to the water as SO₄²⁻ as leaves decay, although atmospheric gases, and the dust on leaf surfaces may also leach SO₄²⁻. Compared to the other parts of the plant, since more SO₄²⁻ was released from

roots in the leaching experiments of this study, it is possible that SO_4^{2-} may be also accumulated in the roots of *Trapa japonica* and is released easily.

In leaching tests of this study, except for the roots, only small amounts of PO_4^{3-} were leached from the different parts of *Trapa japonica*. In addition, PO_4^{3-} was not detected in the pond water during this study, which means that the pond water can be characterized as oligotrophic²². Phosphorus is commonly bound to other elements to form either inorganic or organic compounds. The majority of dissolved phosphorus found in water occurs as orthophosphates¹⁶. Since the water samples collected in the present study were filtered before analysis, the PO_4^{3-} concentrations reported here is soluble phosphorus. Because phosphorus dissolved in water is readily available for uptake by algae and floating vegetation, the concentrations of PO_4^{3-} in the pond water may be low. Phosphorus release from pond sediments is negligible, especially in this pond as it was well oxygenated³⁰.

It was confirmed that $\text{NH}_4\text{-N}$ released from *Trapa japonica* was converted to $\text{NO}_3\text{-N}$ via $\text{NO}_2\text{-N}$ by natural oxygenation in another pond from autumn to winter¹⁷. This result is attributed to seasonal fluctuations in DO concentrations in the pond water; since atmospheric aeration at the gas-liquid interface is affected by water temperature, oxygen supply from the atmosphere into a water body is accelerated when water temperatures are low, resulting in seasonal fluctuations in DO concentration in a waterbody¹⁰.

In the present study, however, DO concentrations in the investigated pond did not fluctuate markedly during the investigated period. It is because, in part, oxygen may be supplied into water of the pond by plants, such as *Trapa japonica*, in August when photosynthesis has been active, while the DO concentration in the water decreases due to high temperatures. Thus, the seasonal changes of $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ reported in the previous reference were not observed in this study (NH_4^+ ; $P > 0.350$, NO_3^- ; $P > 0.07$).

Except for June, Ca^{2+} and Mg^{2+} concentrations tended to be higher in the pond than in the stream, probably due to leaching from *Trapa japonica* and the leaves of other riparian species, such as *Quercus glauca* adjacent to the pond.

CONCLUSION

Trapa japonica releases a variety of substances into water bodies, including carbon, nitrogen, and ions. The concentrations of these substances in the pond examined in this study were observed to fluctuate seasonally, depending on water temperature, DO concentration, flow rates into the pond, and consumption by aquatic flora and fauna. Flushing and the transport of nutrients downstream due to peak inflows during June to August may also have diluted the concentrations of water quality parameters at the water sampling sites. In numerous previous studies conducted in eutrophic systems, markedly rapid nutrient uptake by *Trapa japonica* has been demonstrated³¹ and the release of nitrogen and phosphorus from decaying aquatic plant was increased in water to which nutrients were added⁷.

The present results conducted under oligotrophic conditions showed that the release rates of phosphorus and nitrogen from *Trapa japonica* were extremely low, and thus that the plant does not contribute to water quality degradation in the pond. Even under oligotrophic conditions,

however, parameters, such as TC, TN, Na^+ , Cl^- and SO_4^{2-} , tended to be absorbed by *Trapa japonica* and then released when the plants became submerged. The amounts of these accumulated substances that were released, was in proportion to the amounts of these compounds that were accumulated by *Trapa japonica*. It is therefore important to identify which substances are accumulated and then selectively released by *Trapa japonica* in order to manage the ponds and lakes colonized by this species. These absorption and release characteristics are known to differ between species, parts of plants, the timing of plant emergence, when the plants were harvested¹³ and the effect of sediment on the regeneration of nutrients²⁰. Furthermore, numerous complex interactions between pH and nutrient cycles have been reported^{5, 6, 8, 29}. Further field and laboratory measurements, therefore, are required.

The growth of *Trapa japonica* in the study pond is not out of control and the species is not associated with excessive leaching of nutrients at this site. This is primarily due to the depth of the pond (<2 m) and the fact that nutrient loads do not favor the growth of this species. Consequently, controlling the water level of a pond by lowering the water level may be one way to control the growth of *Trapa japonica*.

However significant differences in the measurement values were obtained depending on the measurement month and sampling points, significant interactions were observed between each pair of variables. Consequently, further study needs to be undertaken to increase the robustness of the data.

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要約

ヒシ (*Trapa japonica*) は、我が国の河岸や湖沼に広く分布する浮葉植物で、水中に含まれる諸成分の吸収による水質浄化機能が評価されている半面、富栄養的水質環境下では異常繁殖し、その結果、ヒシの分解によって溶出する多量の窒素、リン等の栄養塩類が、水域の富栄養化をさらに促進するという悪循環を招く特徴を持っていることが指摘されている。こうしたヒシの溶出特性は、これまで富栄養的環境下で研究解明が行われてきたが、ごく一般的な非富栄養的水質環境下でのヒシの分解と溶出特性については、不明な点が多く残されている。そこで本研究では、本学近傍に位置し非富栄養的水質環境下にある灌漑用ため池（通称前山池）を対象に、現地水質測定および室内溶出実験によりヒシの初期分解とそれに伴う溶出特性について考察した。

2011年6, 8, 10, 12月に現地20地点で水質資料を採水し水中に含まれる成分濃度を測定した。ヒシの繁殖が認められた8, 10月ではヒシを採取し、葉、莖、実、根の部位に区分けし80℃で48時間の乾燥後、各部位の全炭素、全窒素含有率を測定した後、1gを500CCの蒸留水に投入し（各部位5個のサンプルを用意した）30日間での分解に伴う各部位の重量変化と、水中に含まれる成分濃度を測定した。測定したのは、pH, EC（電気伝導度）、TC（全炭素量）、TN（全窒素量）、陽イオン（ Li^+ , Na^+ , NH_4^+ , K^+ , Ca^{2+} , Mg^{2+} ）、陰イオン（ F^- , Cl^- , NO_2^- , Br^- , NO_3^- , PO_4^{3-} , SO_4^{2-} ）である。

ヒシの全炭素、全窒素含有率はそれぞれ26.6～39.5%, 1.3～3.2%であり、他の植物と比較して全炭素含有率は低く、全窒素含有率は同等もしくはそれ以上であることが解った。また、30日間での重量損失率は8月で10～38%, 10月で11～42%で部位間で有意な差（ $P < 0.0001$ ）が認められたが、重量損失率と溶出成分濃度との間に有意な相関関係は認められず、今回計測した成分以外の物質が溶出した可能性が高いことが示唆された。 K^+ は溶出実験で最も多くヒシより溶出したが、現地水中での濃度は今回の測定成分中最も低く、これは水生生物による消費等によるものと推察された。

非富栄養的水質環境下では、ヒシの分解とその後の成分溶出は様々な要因に支配され、富栄養化の原因となるリン、窒

素の溶出は少ないことが確認された。しかしながら、TC、TN、 Na^+ 、 Cl^- 、 SO_4^{2-} がヒシに蓄積されやすく水中では容易に溶出しやすいことが判明した。このことから、ヒシに蓄積

溶出されやすい成分を特定することが、今後のヒシの管理方法を構築する上で重要であると考えられた。

