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Full Length Research Paper

Effect of pH decline by acidified spring water on the growth of a submerged macrophyte, *Egeria densa (Hydrocharitaceae)* in the Kurohashi River, Japan

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A decline in pH shifts the equilibrium balance of HCO_3^- -free CO_2 towards a regime dominated by free CO_2 in the water. Based on the phenomenon, the effect of different pH values on the growth of a submerged macrophyte *Egeria densa* was investigated in an acidified spring-fed stream in Japan. The growth rate of *E. densa* during its developing period was found to negatively correlate to water pH ($p<0.05$). This suggested that a decline of pH in the stream induced by an acidified spring promotes the growth rate of *E. densa*.

Key words: Submerged macrophyte, *Egeria densa*, acidified spring water, pH.

INTRODUCTION

Spring water has recently been recognized as an important ecological component of regional ecosystems (MOEJ, 2010). One of the important characteristic of spring water is that it is rich in free CO_2 due to the respiration of organisms and it lack of CO_2 consumer in the ground (Hanya and Ogura, 1995), thereby affects aquatic plant ecosystems (Rosenberry, 2000; Demars and Trémolières, 2009).

Several studies reported that both in lotic and lentic environments, the biomass of submerged macrophytes depend on the dissolved inorganic carbon (DIC) concentration (Madsen and Sand-Jensen, 1994; Vadstrup et al., 2006). This indicates that water pH, which subsequently governs the amount of inorganic carbon such as free CO_2 in water, is a key factor in determining the growth of submerged macrophytes.

Since lower pH shifts the equilibrium balance of HCO_3^- and free CO_2 towards a regime were free CO_2 dominate in the water (Allan and Castillo, 2007), we hypothesize that the decline of pH in the stream water induced by the acidified spring inputs, increases the free CO_2 concentration in the water and thereby promotes the growth rate of *Egeria densa*.

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METHODS

Study site

The study site is located in an upstream reach of the Kurohashi River (35°06'57.0N, 136°07'11.2E - 35°07'04.2N, 136°06'51.1E), which is an acidified spring-fed stream flowing into the Lake Biwa, Japan and is abundant with submerged macrophytes, such as *E. densa* Planchon. The Kurohashi River extends for 5.5 km; its catchment area is 6.9 km^2 and the average riverbed slope is approximately 1/1000. The study site stretch is 0.8 km long, with a width ranging from 1 to 2 m. There is a spring-fed pond in an upstream area feeding the stream water (Figure 1), while the upstream reach of the study site also contains several places where spring water surges from the riverbed (Figure 1, St.3 to 4). The main stream at the study site has seven tributaries, but no vegetation growing, one of which supplies spring water to the mid-stream reach (Figure 1). The upstream reach of the Kurohashi River is used as an irrigation channel for agricultural purposes and had the streambed inhabited with submerged macrophytes. It is dredged annually to maintain the flow condition of the channel. During the study period, dredging was carried out on 5 July, 2009 and 4 July, 2010. After those periods macrophytes were removed,

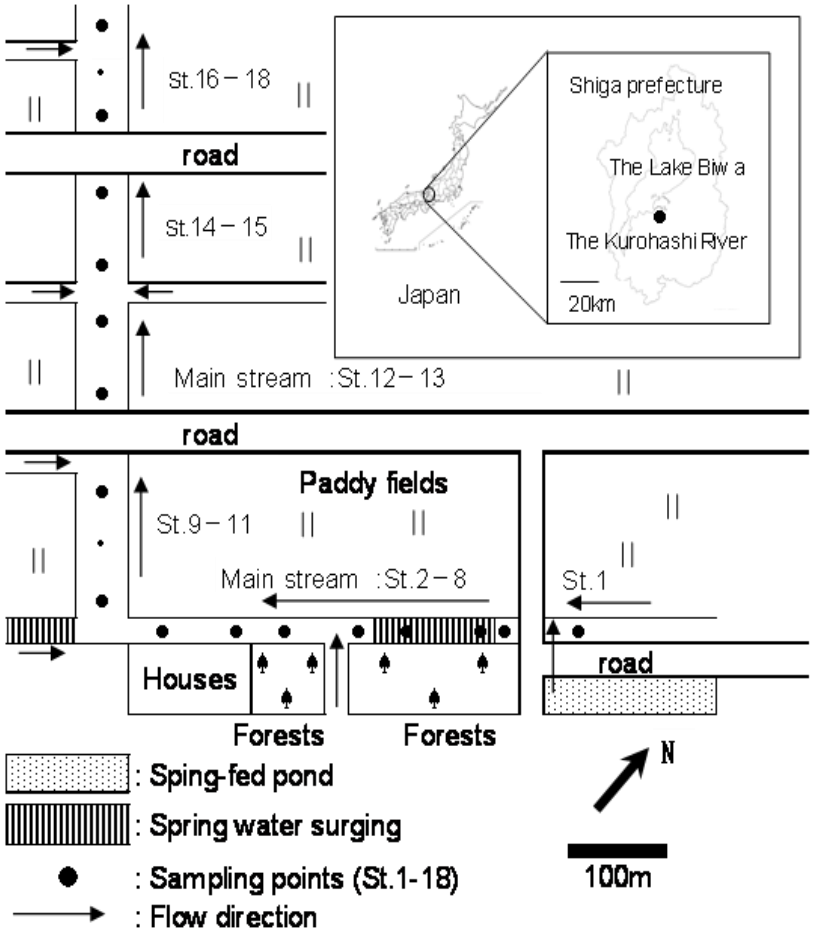

Figure 1. The study site and sampling points. Dots indicate sampling points. There is a spring-fed pond in an upstream area shown as square with dots. Hatch with vertical lines indicate places where spring surges from the riverbed.

but re-grew rapidly.

In-situ sampling

Sampling points were located approximately every 50 m at 16 points (St.2 to 17) in the Kurohashi River. Samplings were carried out in autumn (September to October, 2009), winter (November to December, 2009) and spring to summer (May to early July, 2010). In each sampling point, macrophytes, water and sediment samples were collected. To assess biomass of *E. densa* one quadrat, 30 cm × 30 cm for each sampling point was settled to collect plants. In each sampling date, physical and chemical water parameters such as pH, temperature, dissolved oxygen (DO) and electric conductivity (EC) were measured by the Onsite Aqua Sensor Integrated System (OYO Corporation) at different daytimes; during the morning, at the midday and in the afternoon. The obtained data were averaged at each sampling point.

Laboratory analysis

Macrophytes, water and sediment samples were stored in cool boxes and immediately brought to the laboratory, where they were dried at 80°C using a convection oven, (NDO700, TOKYO RIKAKIKAI Co., Ltd.) for more than 3 days until no further weight change was detected.

The sediment fractions of different particle sizes were measured based on the protocol of the American Society for Testing and Materials (ASTM D422-63, 2007). Total nitrogen content (T-N) of the sediment was analyzed by the Vario Micro Cube (Elementar Co., Ltd.) and its total phosphorus content (T-P) was determined with the ascorbic acid method after digestion using the perchloric acid method (APHA, 2005). The biomass of the macrophyte samples was also measured for each species.

The ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N) and phosphate (PO_4-P) in the water were analyzed by an automatic analyzer (TRAACS800, BL TEC K.K.). The concentrations of ammonium were determined by the phenate method and the concentrations of nitrites by the sulfanilamide-NED dihydrochloride colorimetric method (APHA, 2005). Nitrate concentrations were determined by the cadmium reduction method and phosphate concentrations were determined by the ascorbic acid method (APHA, 2005).

Data analysis

The growth rate of *E. densa* was calculated by the Equation (1), taking into account changes in the biomass of the populations

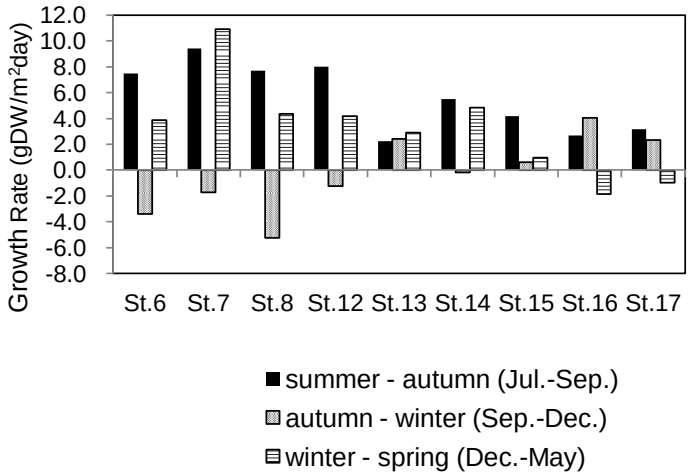

Figure 2. Seasonal changes in the growth rates of *E. densa* in the Kurohashi River, Japan during the study period.

between the different seasons: summer to autumn (5 July to 20 September, 2009), autumn to winter (21 September to 6 December, 2009) and winter to spring (7 December, 2009 to 5 May, 2010). A dredging procedure was carried out on 5 July, 2009. Hence the biomass was zero on that day.

$$GR\ (gDW/m^2day) = Biomass\ (gDW/m^2) / Days\ (day) \quad (1)$$

Where, "GR" is the growth rate, "Biomass" represents dry weight of plants measured on each sampling day and "Days" represents the number of days between the present and former sampling dates.

In the statistical analysis, Student's t-test was carried out with Excel 2007 (Microsoft Corporation) and Spearman rank correlation coefficients were determined with Excel Statistics 2008 (SSRI Co., Ltd.).

RESULTS AND DISCUSSION

Seasonal changes in the growth rate of *Egeria densa*

E. densa was the dominant species in the study site, distributing from mid- to downstream reaches (St. 6 to 17 except for St. 9 to 11) during the study period.

The growth pattern of *E. densa* differed between seasons and reaches (Figure 2). In the downstream reach, the growth rates were positive at all the sampling points from summer to autumn while in the mid-stream reach they were negative from autumn to winter except for St. 13, conversely the same in the downstream reach the growth rates were negative from winter to spring except for St. 15 (Figure 2).

Growth pattern of *E. densa*

Haramoto and Ikusima (1988) reported that the biomass of *E. densa* in the Lake Kasumigaura at eastern Japan peaked in October, then drastically declined, forming a thick mat on the bottom when water temperature decreased in February. Shoots elongated up to the water surface when the water temperature reached 15°C. In the present study, a similar growth pattern was obtained in the mid-stream reach, while it was rather different in the downstream reach, elongating shoots even in winter. The mean water temperature during winter was close to 15°C (St. 6-8, 12-14=15.5±0.7-16.6±0.1°C, St. 15-17= 15.2±0.8-15.3±0.7°C), which inhibited the active growth. Negative growth rates in the mid-stream reach seem to be attributable to the partial dying off due to lower temperature in winter.

The growth of submerged macrophytes is also dependent on light availability (Barko et al., 1986). The shoot located near the riverbed, probably did not receive sufficient light because of inhibition by large foliage at upper layer of plant stands. Thus in the mid-stream reach *E. densa* had the lowest biomass.

In contrast, the growth rates were positive at St. 16 and 17 from autumn to winter. Biomass values at these sampling points were very low in autumn and thus plants could have still kept growing in late autumn to early winter in order to prepare for the quick elongation of shoots for the next growing season. The negative growth rate at St.16 and 17 from winter to spring was probably due to the lack of enough storage in propagules in winter.

Correlations between the growth rate and environmental factors

On the one hand, in the periods when vegetation did not grow, the average water depth in upstream reach (St.2-5), mid-stream reach (St.6-14) and downstream reach (St.15-17) were around 20, 20 and 25 cm, respectively and the flow velocity in upstream reach, mid-stream reach and downstream reach were 20 to 30, 20 to 70 and

Table 1. Spearman rank correlation coefficients between the growth rate of *Egeria densa* and environmental factors in the Kurohashi River, Japan during the study period.

Environmental factor	Growth rate		
	Summer to autumn	Autumn to winter	Winter to spring
pH	-0.70*	0.82**	-0.77*
Temperature	-0.10	-0.79*	-0.71*
DO	-0.88**	0.90**	-0.77*
EC	-0.63	0.65	-0.39
NO_3-N	0.20	-0.57	-0.20
NO_2-N	0.30	0.47	-0.39
NH_4-N	0.19	-0.25	0.23
PO_4-P	0.09	-0.55	0.46
Particle diameter(d_{50})	0.22	-0.40	-0.36
T-N content of soil of bottom sediment	0.25	0.30	0.26
T-P content of bottom sediment	-0.10	0.05	0.24

** $p<0.01$, *$p<0.05$.

20 to 40 cm/s, respectively. On the other hand, when the biomass peaked, the average depth of those reaches were around 15, 30 and 40 cm, respectively and water flow were around 10, less than 10 to 40 and 20 to 40 cm/s, respectively.

The growth rate of *E. densa* was negatively correlated to pH and DO in the periods of summer to autumn and winter to spring, while it was negatively correlated with water temperature in the period of winter to spring (Table 1). Spring water generally lacks in DO because oxygen is consumed by respiration in the ground (Hanya and Ogura, 1995), implying that increasing inputs from spring water positively influence the growth rate of *E. densa*. In the main stream points fed by the spring water (St. 6 to 17), mean temperature was significantly lower during spring, than in the tributary (Student's t-test; $p<0.01$). This implies that the difference in water temperature was affected by the quantity of spring water inflow.

From autumn to winter, the growth rate positively correlated to pH and DO and negatively correlated to water temperature, which implies that the growth rate inversely correlated to the quantity of acidified spring water flowing into *E. densa* stands. This may be attributable to the negative growth rates of the large biomass in the mid-stream reach, which grew well in autumn under the low pH condition induced by acidified spring water.

Effect of spring water on the growth of *E. densa*

Titus et al. (1990) assumed the vigorous macrophyte growth at high DIC levels in low pH lakes on the basis of the experimental result. The present study, where low pH of the stream water induced by acidified spring increased the growth rate of *E. densa*, supports this hypothesis.

Approximately 50% of macrophytes can use HCO_3^- for photosynthesis (Yarrow et al., 2009) providing H^+ in order to create acid spots where free CO_2 are generated from HCO_3^-, which is called "proton pumps" (Lara et al., 2002). There are several researches reporting that the growth of macrophytes at lower pH is greater than at higher pH with constant DIC levels (Sand-Jensen, 1983; Titus et al., 1990). Therefore, these previous studies supported that the growth rate of *E. densa* may be promoted by low pH in the stream surged with acidified spring water.

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