

Effects of Epiphyte Density on Seagrass Leaf Turnover Rate

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An experimental investigation on the effects of epiphyte density on leaf turnover rate was carried out on four temperate seagrass species: *Zostera japonica*, *Zostera marina*, *Zostera asiatica*, and *Phyllospadix iwatensis*. The four seagrass species were cultured in outdoor tanks under ambient conditions and leaf turnover rates were measured with the presence and absence of epiphytes. The leaf turnover rates of *Z. japonica*, *Z. asiatica*, and *Z. marina* when epiphytes were removed were significantly lower, but the same seagrass species exhibited faster leaf turnover rates under the control condition. The leaf turnover rate of *Z. japonica*, *Z. marina*, and *Z. asiatica* within the control and experimental conditions was significantly affected by the changes in the epiphyte density, while no significant effect was detected in *P. iwatensis*. This study shows that high epiphyte density enhanced high leaf turnover rates exhibited by *Zostera* species, as the adaptive mechanism against epiphytism.

Key Words: epiphytism, leaf turnover, *Phyllospadix iwatensis*, *Zostera asiatica*, *Zostera japonica*, *Zostera marina*

INTRODUCTION

Seagrass meadows have been considered as one of the most productive of the marine ecosystems, contributing significantly to the productivity of shallow coastal areas of both temperate and tropical waters (McRoy & McMillan 1977; Zieman & Wetzel 1980). Seagrass epiphytes are an important component of these highly productive ecosystems, often contributing greater than one-third to the total above ground biomass (Penhale 1977; Heijs 1984; Tomasko & Lapointe 1991) and as much as 30% to the combined seagrass/epiphyte productivity (Penhale 1977; Morgan & Kitting 1984; Heijs 1984, 1985, 1987).

In addition to being an important component of the seagrass community, epiphytes could also be detrimental to seagrasses. As an example, epiphytes can create physical

barriers to light absorption (Losee & Wetzel 1983; Dalla Via et al. 1998; Brush & Nixon 2002), interfere with the uptake of nutrients, reduce oxygen diffusion rate, or a combination of these factors that may act on seagrasses (Sand-Jensen 1977; van Montfrans et al. 1984; Sand-Jensen et al. 1985).

Various factors affect the turnover rates of seagrass leaves. Physico-chemical factors affecting leaf turnover rates include nutrient availability, temperature, salinity, light, and others. Epiphyte density has also been implicated as causal factor for variation in leaf turnover rates (Borowitzka & Lethbridge 1989).

Few studies have evaluated the effect of leaf turnover rate on epiphyte density because of the difficulty in manipulating leaf turnover rates without altering other factors (Peterson et al. 2007). Because of this difficulty, most authors have chosen to manipulate epiphyte density

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rather than attempting to experimentally control leaf turnover over rates.

MATERIALS AND METHODS

Collection of seeds

Seed-bearing shoots of *Zostera japonica*, *Zostera marina*, *Zostera asiatica*, and spathes of *Phyllospadix iwatensis* were collected during the months of July and August 2004. The seed-bearing shoots of *Z. marina*, *Z. asiatica*, and *P. iwatensis* were collected at Aininkap, Akkeshi Bay, Hokkaido, Japan (43° 00' N, 144° 51' E), while those of *Z. japonica* were collected at the Akkeshi-ko estuary (43° 01' N, 144° 53.4' E). After the collection of the seed-bearing shoots, these were brought to Akkeshi Marine Station and were placed inside the outdoor running seawater tanks. The shoots were allowed to release their seeds in the tanks. The seeds were then separated from the detrital remains of the reproductive shoots/spathes through several steps of screening and winnowing, and held in ambient flowing seawater for several months prior to actual culture according to Granger et al. (2000).

Culture of seeds

The seeds of *Zostera japonica*, *Zostera marina*, *Zostera asiatica*, and *Phyllospadix iwatensis* were planted in the third week of April 2005. Sandy sediments collected from Aininkap, Akkeshi Bay were used for planting *Z. marina* and *Z. asiatica* seeds, while the muddy sediments collected from Akkeshi-ko estuary were used for planting *Z. japonica* seeds. About 24 seeds each of *Z. japonica*, *Z. marina*, and *Z. asiatica* were buried at 7 cm depth in individual plastic planter (26.5 cm x 26.5 cm). On the other hand, the seeds of *P. iwatensis* were properly attached and anchored in the plastic nets. The plastic nets with the seeds were placed on the rocks because the seeds will not grow if buried under the sediment. The seeds were then allowed to germinate in outdoor tanks with running seawater from Akkeshi Bay under conditions of ambient light and temperature. Seedlings of the four seagrass species began to germinate in the third week of May 2005. After this time, the first seedling leaf of all species began to appear.

Experimental set-up

The experiment was conducted during the month of August 2005. Prior to the commencement of the presence-absence of epiphytes experiment, twenty (20) shoots of each seagrass species were selected. Selection of shoots includes the similarity in the size of leaves (i.e., length and

width) for each species. Each shoot in both tanks for all seagrass species were marked using tiny thin sticks with attached vinyl tapes on the tips and then numbered. Two treatment tanks were set-up for each species- these tanks were referred to as the control and experimental tanks. Each tank had ten shoots for each seagrass species. Water depth in both tanks (control & experimental) was 50 cm. Culture tanks were maintained with running seawater from Akkeshi Bay, with similar salinity and temperature (29-32‰ and 16-21° C during the experiment), and light intensity (1200-1700 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$).

Measurement of leaf turnover rate

Ten shoots of every seagrass species were haphazardly selected for leaf turnover measurement. The middle of the sheath of each shoot was punched with a needle. These shoots were harvested 30 days after leaf marking (Sand-Jensen 1975). The turnover time or the leaf plastochrone interval (PL, day) was calculated based on the formula of Short and Duarte (2001):

$$P_L = \frac{T_1 - T_0}{N}$$

where:

T_1 = final time in days

T_0 = initial time in days

N = number of new (unmarked) leaves within time period

In order to determine the effect of epiphyte density on leaf turnover rates, the seagrass shoots in the control tanks remained untreated (i.e., no removal of epiphytes throughout the duration of the study). In contrast, shoots growing in experimental tanks were cleaned everyday by gently removing the epiphytes from the leaves by hand.

Epiphyte density

The density of epiphytes on seagrass leaves was measured as chlorophyll a after the experimental period. Ten shoots of every seagrass species were collected from both the control and experimental tanks. Epiphytes were gently removed from all leaf blades (3-4 blades) using soft brush. The epiphytes suspensions were filtered (Whatman GF/C), and the filtrate was analyzed fluorometrically after extraction in N,N-Dimethylformamide (DMF). The performed extraction and calculation of chlorophyll a were done using the method of Aran & Collins (1992). The epiphyte density per unit leaf area was expressed as $\mu\text{g Chl a cm}^{-2}$.

Environmental variables

The environmental variables, namely water temperature, salinity, and light intensity, were determined in both control and experimental tanks. Water temperature, salinity, and

light intensity in both tanks were measured daily during the experimental period. Salinity and temperature were measured using a salinity/temperature meter (Central Kagaku Corp. Model UC-78), while light intensity (PAR photon flux density or PPF) was measured at the seawater surface, just above the cultured seagrasses, using a submersible fiber quantum sensor (calibrated against a LI-COR Quantum Sensor, Type LI-190). All measurements were taken at 9-10 o'clock in the morning.

Analyses of data

The difference in leaf turnover rates between experimental and control condition for each seagrass species was analyzed using two-way analysis of variance (significance level: p of 0.05). A simple linear regression was used to determine the effect of epiphyte density on leaf turnover rates for each seagrass species. All datasets were found to meet assumptions of normality and equal variance. The analyses were performed using the SPSS, Inc. (v8.0).

RESULTS AND DISCUSSION

Environmental factors

The mean values of water temperature in control tanks ranged from 17-21° C for *Z. japonica* and *Z. marina*, 18-22° C for *Z. asiatica*, and 17-22° C for *P. iwatensis*. In the experimental tanks, water temperature ranged from 17-22° C for *Z. japonica*, 16-21° C for *Z. marina*, 19-22° C for *Z. asiatica*, and 18-22° C for *P. iwatensis*. The salinity in control tanks ranged from 30-31psu for *Z. japonica* and *Z. marina*, 31-32psu for *Z. asiatica*, and 30-31psu for *P. iwatensis*. In the experimental tanks it ranged from 30-31psu for *Z. japonica*, 29-30psu for *Z. marina*, 30-31psu for *Z. asiatica*, and 31-32psu for *P. iwatensis*. The light intensity in the control tanks ranged from 1210-1700 for *Z. japonica*, 1200-1700 for *Z. marina*, 1190-1670 for *Z. asiatica*, and 1200-1680 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for *P. iwatensis*. On the other hand, light intensity in the experimental tanks ranged from 1190-1700 for *Z. japonica*, 1220-1710 for *Z. marina*, 1200-1675 for *Z. asiatica*, and 1220-1710 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ for *P. iwatensis*. The environmental variables measured between the control and experimental tanks for each seagrass species showed no significant difference (two-way ANOVA; $F = 1.000$; $df = 1, 174$; $p > 0.05$).

Leaf turnover rates

The mean leaf turnover rates measured between the control and experimental tanks vary significantly for each seagrass species (two-way ANOVA; $F = 49.000$; $df = 1, 72$; $p < 0.05$).

Without epiphytes' removal (control condition), the leaf turnover rates of *Zostera* species were significantly high, while with constant removal of epiphytes, leaf turnover rates were low (Figure 1). On the other hand, the leaf turnover rates of the three species of *Zostera* (*Z. japonica*, *Z. marina*, and *Z. asiatica*) were significantly affected by the change in the epiphyte density with coefficients of determination (r^2) being 0.94 (Control: $F = 121.752$; $df = 8$; $p < 0.05$) and 0.91 (Experimental: $F = 118.232$; $df = 8$; $p < 0.05$) for *Z. japonica*, 0.73 (Control: $F = 21.461$; $df = 8$; $p < 0.05$) and 0.77 (Experimental: $F = 27.529$; $df = 8$; $p < 0.05$) for *Z. marina* and 0.51 (Control: $F = 26.745$; $df = 8$;

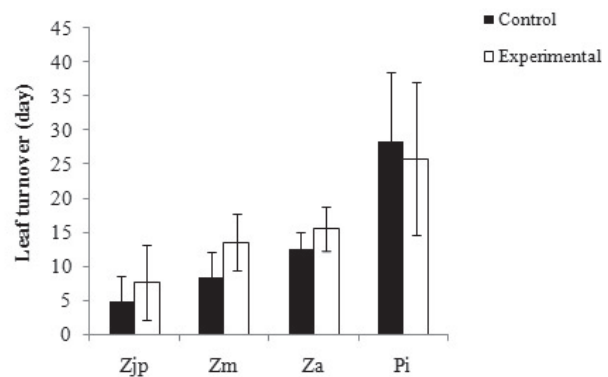


Figure 1. Leaf turnover rates of *Zostera japonica*, *Zostera marina*, *Zostera asiatica*, and *Phyllospadix iwatensis* under the control and experimental conditions.

$p < 0.05$) and 0.57 (Experimental: $F = 10.907$; $df = 8$; $p < 0.05$) for *Z. asiatica*, respectively (Figures 2 & 3). Significant treatment main effects or treatment x species interactions on the measured leaf turnover rates were not observed. No significant effect was detected for *P. iwatensis* leaf turnover rates.

Several variables have been known to affect the leaf turnover rates in seagrasses. These variables include both environmental and biological variables. The environmental variables measured between the control and experimental tanks were negligible as the values did not show any significant difference. Other variables such as the amount of nutrients are also important in affecting leaf turnover rates (Hemminga & Duarte 2000) but measurement was not done due to unavailability of materials in the working station. However, biological variable such as epiphyte density varies significantly in *Z. japonica*, *Z. marina*, and *Z. asiatica*. This was further supported by the result of the linear regression in which a significant difference was detected in leaf turnover rates versus epiphyte loads among the three species of *Zostera*. This study then suggests that epiphyte density has affected the leaf turnover rates which were mostly evident in *Zostera* species.

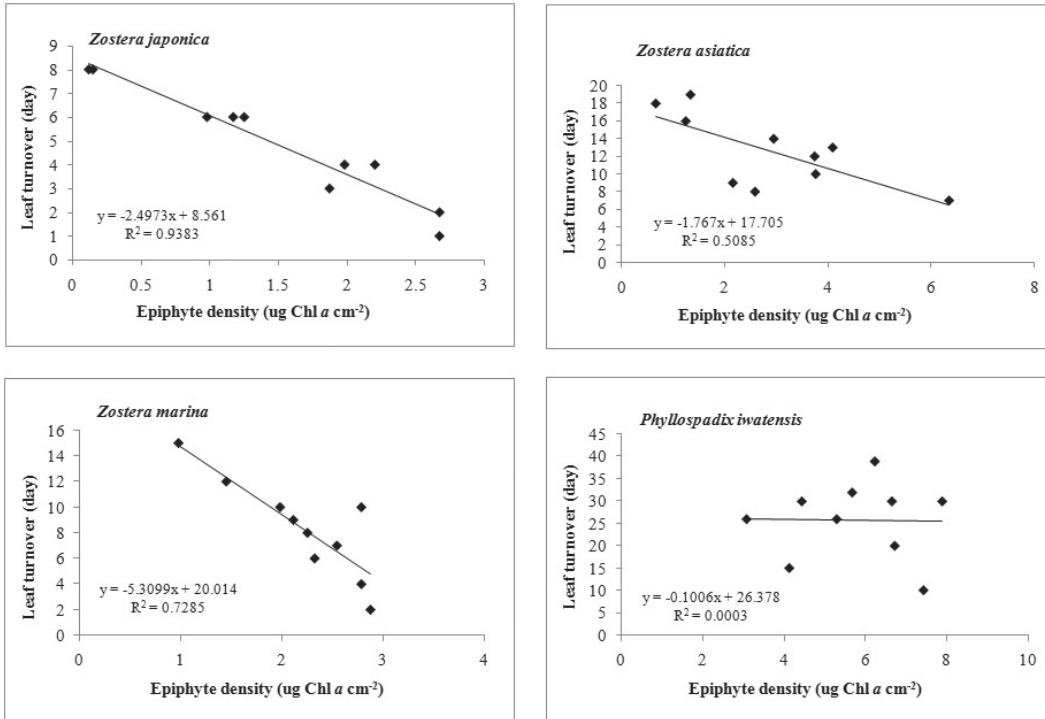


Figure 2. Effects of epiphyte density on leaf turnover rates of *Z. japonica*, *Z. marina*, *Z. asiatica*, and *P. iwatensis* under the control condition.

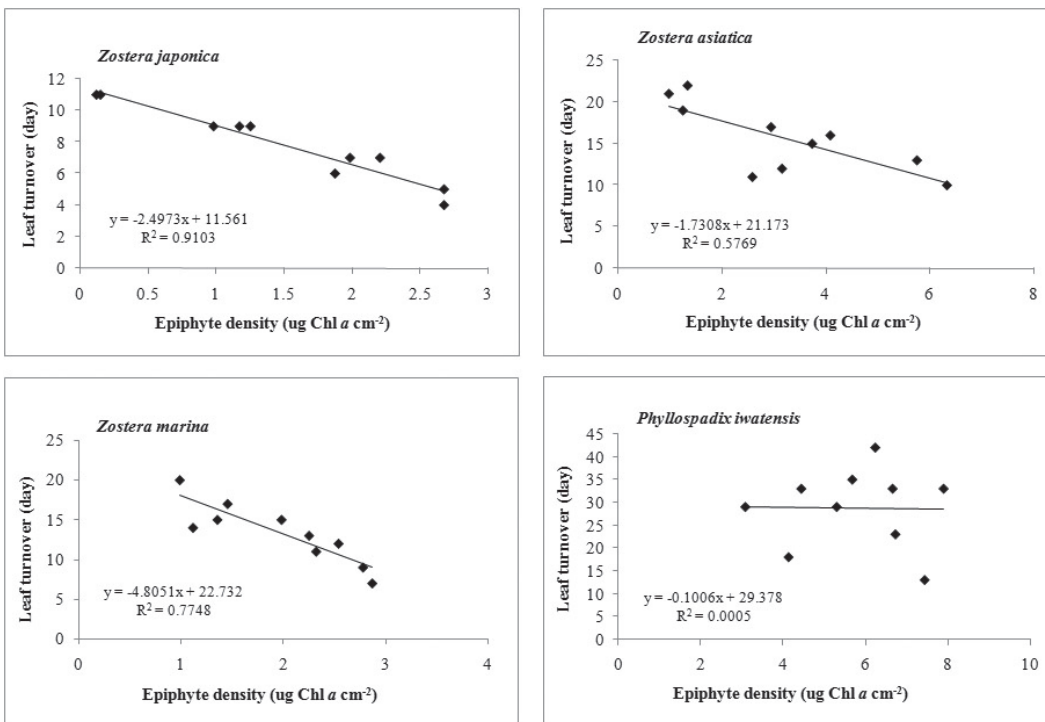


Figure 3. Effects of epiphyte density on leaf turnover rates of *Zostera japonica*, *Zostera marina*, *Zostera asiatica*, and *Phyllospadix iwatensis* under the experimental condition.

The high coefficients of determination (r^2) for *Zostera* species would mean that the degree of dependency of leaf turnover rates to the change in epiphyte density is high. Although results show significant effect of epiphyte density on leaf turnover rates, high turnover rate was observed in the control tanks without removal of epiphytes. This result further indicates that *Zostera* species have developed an adaptive mechanism that would allow these seagrass species to survive despite the presence of epiphytes. This mechanism of fast leaf turnover rate limits the time for colonization of epiphytes (Borowitzka & Lethbridge 1989, Kendrick & Lavery 2001) that would minimize its effects on seagrass growth and production. As an example, in Roscoff, France and Oresund, Denmark, a reduction of epiphyte biomass was observed as a result of an increased rate of leaf renewal (Jacobs et al. 1983, Borum et al. 1984). Unlike corals and algae which exude chemical substances to tear off epithelium (Mukai 1990, Borowitzka & Vesik 1978, Moss 1982), seagrasses have not developed evolutionally such anti-epiphyte mechanism. Hence, fast leaf turnover may be a good strategy to counteract epiphytism in seagrasses.

In contrast to *Zostera* species, the leaf turnover rate of *P. iwatensis* was low in both conditions. The slow turnover rate of this species can be attributed to its low growth rate which also resulted to longer leaf life span (Sasil-Orbita 2006). The slow growth rate of *P. iwatensis* can be a good survival mechanism because it is always growing on rocky shore in surf zones and suffering from wave disturbances of high energy. As a consequence, this species needs to have tough leaves which have high cost. Importantly, epiphytism could only have a minimal effect on this species because of its being dark-adapted (Sasil-Orbita 2006). In this case, there would be no competition between *P. iwatensis* and its epiphytes in terms of light.

The colonization of epiphytes and superiority for light competition has developed strategies in seagrasses. These strategies could be a defense or protection of seagrasses against epiphytes and its survival. Moreover, these strategies are needed for the high rate of primary production (e.g., high leaf turnover rate) in seagrasses.

SUMMARY AND CONCLUSION

This study has clarified the effect of epiphyte density on leaf turnover rates in four temperate seagrass species, namely *Z. japonica*, *Z. marina*, *Z. asiatica*, and *P. iwatensis* found in Akkeshi Bay and Akkeshi-ko estuary, Hokkaido, Japan.

The results of the present study suggest that high leaf turnover rate could be part of the mechanisms controlling epiphytism in seagrasses, or may be due to a possible set of traits, i.e., high growth rate, short longevity, and high leaf production. This fast leaf turnover mechanism was observed in the three species of seagrasses, namely *Z. japonica*, *Z. marina*, and *Z. asiatica*.

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