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Relationship between epiphytes and the photosynthetic activity of temperate seagrasses

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Abstract. The relationship between epiphytes and the photosynthetic activity of *Zostera japonica*, *Zostera marina*, *Zostera asiatica* and *Phyllospadix iwatensis* was investigated. The epiphyte component of the seagrass leaves was composed primarily of diatoms and red calcareous algae. The epiphyte density was highest in the oldest region of the leaves and the oldest leaf in a shoot. The relationship between epiphytes and the photosynthetic activity of seagrasses is inversely proportional. The highest photosynthetic activity was observed in the younger leaves with low epiphyte density and vice-versa. This result indicates that the colonization of epiphytes in seagrasses is age-dependent.

Key Words: Epiphytes, photosynthetic activity, age, temperate seagrasses.

Introduction. The photosynthetic activity of seagrasses varies with some physical factors such as temperature, salinity, light condition and others. Besides, age of leaf and biological interactions with epiphytes and epifauna are also substantial on the photosynthesis of seagrasses. The distribution of epiphytes on seagrass leaves is influenced by the relative age of different sections of each leaf's surface (Borowitzka & Lethbridge 1989). For instance, Heijs (1985) observed that the epiphyte cover on *Thalassia hemprichii* was higher in the tip region of the leaves, as well as in the oldest leaf of a shoot. Similar gradients of within leaf and/or between leaves epiphytic algal distributions have been reported for *Posidonia oceanica* (Van der Ben 1971), *Zostera marina* (Jacobs et al 1983; Mazzella & Alberte 1986), *Heterozostera tasmanica* (Bulthuis & Woelkerling 1983) and *Amphibolis antarctica* (Bramwell & Woelkerling 1984). In this context, the colonization of epiphytes on seagrass leaves is age-dependent. On the other hand, the epiphyte colonization alters the light microenvironment through shading, therefore notably reducing light availability to the leaf surface (Sand-Jensen 1977; Sand-Jensen et al 1985; Drake et al 2003). As an example, Mazzella & Alberte (1986) found out that the old leaf of *Z. marina*, which had a higher amount of epiphytes, had a lower photosynthetic rate (P_{max}). Besides, the tip region of *Zostera capricorni* leaf had a lower photosynthetic activity, which was due to the high amount of epiphytes (Ralph & Gademann 2005). This paper tried to investigate the relationship between epiphytes and the photosynthetic activity of the four temperate seagrasses (*Zostera japonica*, *Zostera marina*, *Zostera asiatica* and *Phyllospadix iwatensis*) in Akkeshi Bay and Akkeshi-ko estuary, Hokkaido, Northern Japan.

Material and Method. In July 2003, five shoots of *Z. marina, Z. asiatica* and *P. iwatensis* were collected randomly in *Zostera* and *Phyllospadix* meadows at Aininkap (43º00´N, 144º51' E), Akkeshi Bay, Hokkaido, northern Japan, while the shoots of *Z. japonica* were collected at Akkeshi-ko Estuary (43º01´ N, 144º53.4´ E). In this study, all the seagrass species were collected only on the population in the upper subtidal zone (0 - 1 m), during low tide. The collected shoots of seagrasses were placed inside the plastic nets, placed in plastic pails with seawater and brought to the laboratory for analysis. The amount of epiphytes on seagrass leaves was measured as chlorophyll *a* content. Four kinds of leaves were examined: Leaf 1 was the youngest and was in a central position in the shoot, Leaf 2 was the second youngest, Leaf 3 was an intermediate leaf, and Leaf 4 was the oldest and was located in the outermost position in the shoot and possessed the highest epiphyte cover. In order to have a detailed analysis of the epiphyte cover, each leaf was equally divided (but not cut) into three sections such as the base, middle and tip. These sections were used for the photosynthetic activity measurements for the seagrasses after the removal of epiphytes. The epiphytes were removed by scraping the leaves (both sides) with small soft brush. The scraping procedure did not alter the leaves' photosynthetic performance, nor damage the leaves as detected by light microscopy. The epiphytes suspension was 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* content were done using the method of Aran & Collins (1992).

The amount of epiphytes on each section in a leaf was calculated as µg Chl *a* per leaf surface area (cm^{-2}). The chlorophyll *a* fluorescence measurements for *Z. marina*, *Z. asiatica, Z. japonica* and *P. iwatensis* were carried out after the removal of epiphytes. By using a Diving-PAM (Walz GmbH, Effeltrich, Germany), the effective quantum yield of PS II (Φ_{PSII}) was measured. The effective quantum yield (Φ_{PSII}) was determined during steady-state photosynthesis in the light using the saturating-light method as ($\Phi_{PSII} = \Delta F / F'm = F_t{´}] / F'm$, where $F'm$ designates maximal fluorescence during a 0.8 s saturating light pulse, and $F_t{´}$ is the steady state level immediately prior to the flash (Genty et al 1989).

The variation in the epiphyte density among leaves of each seagrass species was analyzed using one-way analysis of variance (ANOVA), at significance level (P) of 0.05. The Tukey's multiple comparison tests was performed to identify which treatments were different. To determine the effect of epiphytes on the photosynthetic activity, the Pearson Product-Moment Correlation Coefficient was used (significance level of 0.05). All datasets were found to meet the assumptions of normality and equal variance. These statistical analyses were performed using SPSS, Inc. (v8.0).

Results and Discussion. The epiphyte community on *Z. japonica*, *Z. marina*, *Z. asiatica* and *P. iwatensis* leaves was dominated by diatoms in the genera *Amphora*, *Cocconies*, *Synedra*, *Navicula*, *Nitzschia, Melosira, Rhopalodia* and by encrusting calcareous and macrophytic red algae in the genera *Ceramium* and *Polysiphonia*. On the other hand, *P. iwatensis* was found to have the highest coverage of the calcareous red algae (*Pneophyllum zostericola*). Occasionally, the cyanobacteria *Lyngbya* spp. and the brown alga *Ectocarpus* sp. were found in the seagrass samples. The abundance of these microalgae on seagrass leaves had also been observed in some studies (e.g. Jacobs et al 1983; Mazzella & Alberte 1986; Hamamoto et al 1996). The diatoms also dominated the epiphyte community on the seagrass leaves of *Z. marina* in Akkeshi-ko estuary, and found to be responsible for the increase of diatom abundance in the water column (Kasim 2006). Importantly, this specific group of microalgae (diatoms) was observed to affect the photosynthetic rate of *Z. marina* leaves by acting both as barrier to carbon uptake and light intensity (Jacobs et al 1983). In constrast, Mazzella & Alberte (1986) could not detect any detrimental effect of the epiphytes (dominated by diatoms) on the photosynthesis of *Z. marina*. The epiphyte density on *Z. japonica*, *Z. marina*, *Z. asiatica* and *P. iwatensis* leaves showed a clear apico-basal zonation and the result was significant except for *Z. japonica* (Table 1).

The higher epiphyte density was found in the apical region, while the lower epiphyte density was found in the basal region in all leaves (Figure 1). Additionally, when the data were pooled to get the mean epiphyte density for each individual leaf, it showed that the oldest leaves (Leaf 4) had the highest epiphyte density, and it varied significantly with the other leaves for each seagrass species, except for *Z. japonica* (Figure 2). The higher epiphyte density in the middle and tip region of the leaves, and the oldest leaves in the shoot could be attributed to the presence of light which is of greatest amount on top of the canopy. Enriquez et al (2002) found out that a leaf can experience irradiance gradients, which change by three orders of magnitude over the leaf's lifetime. As a consequence of this light gradient, the abundance of epiphytes along the leaves and among leaves in a shoot would also vary. The chlorophyll *b* was undetectable in the epiphyte community, indicating that the epiphyte removal procedure did not damage the seagrass epidermal cells which might alter the result of the seagrass photosynthetic activity.

Table 1

Analysis of variance (2-way) of epiphyte density (µg Chl *a* cm^{-2}) by tissue age along the leaf axis and among leaves in a shoot of *Zostera japonica*, *Zostera marina*, *Zostera asiatica* and *Phyllospadix iwatensis* (mean ± SE; n = 36).

Species	*Variable*	*Source*	*d.f.*	*MS*	*F*	*p*
Z. japonica	Epiphyte density	Within leaf	2	0.098	0.125	0.53
		Among leaves	3	0.133	0.462	0.71
Z. marina		Within leaf	2	3.817	8.371	<0.001
		Among leaves	3	9.498	20.829	<0.001
Z. asiatica		Within leaf	2	0.535	1.807	0.005
		Among leaves	3	2.432	8.217	<0.001
P. iwatensis		Within leaf	2	7.673	15.371	<0.001
		Among leaves	3	4.179	8.371	<0.001

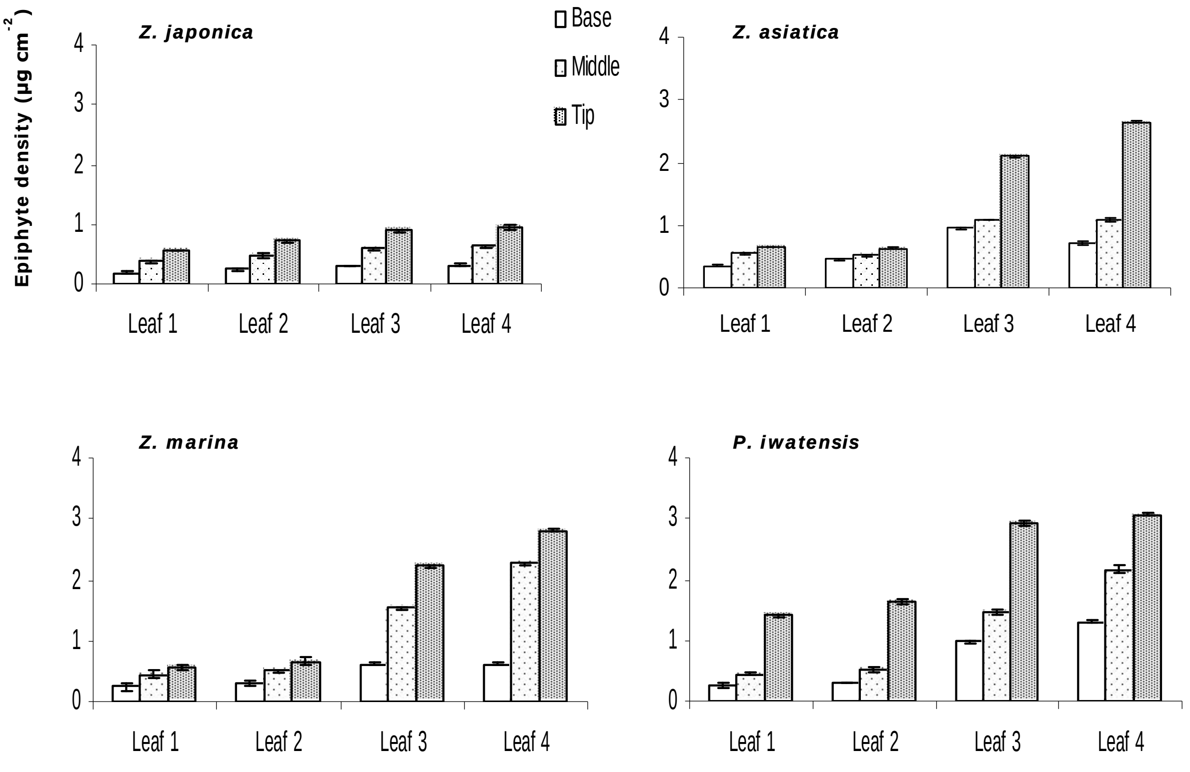

Figure 1. The epiphyte density measured within leaves of the four temperate seagrass species (mean ± SE; n = 36).

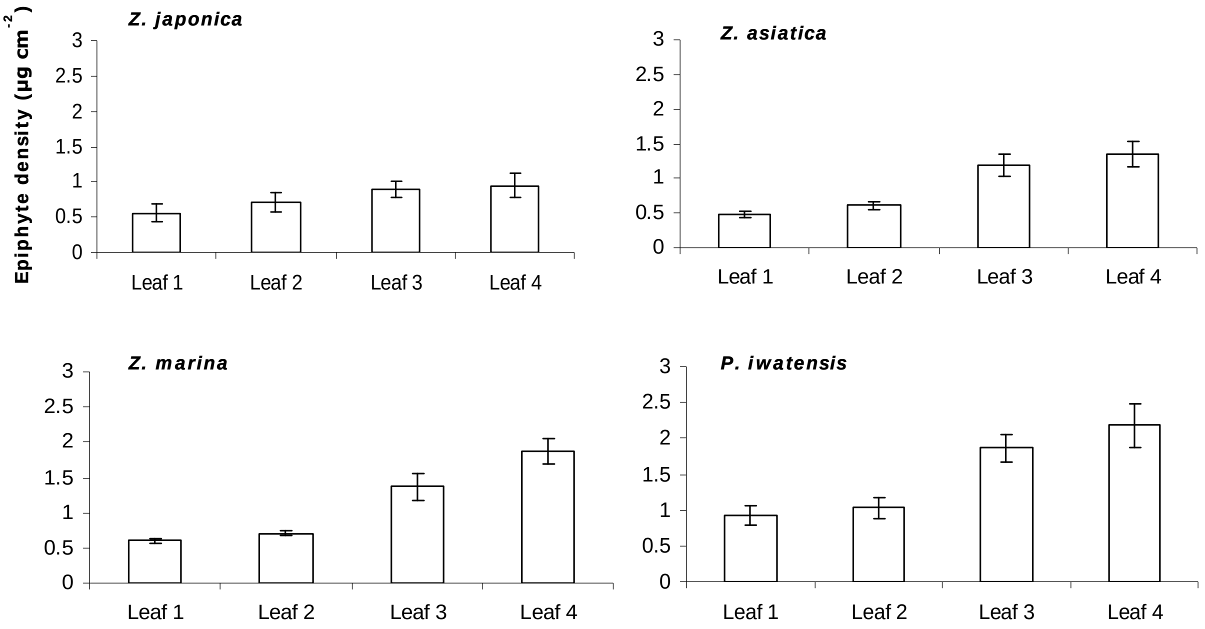

Figure 2. The epiphyte density measured among leaves of the four temperate seagrass species (mean ± SE; n = 36).

The PAM fluorometry performed on seagrass leaves showed a reduced effective quantum yield (Φ_{PSII}) on the oldest region (tip) of the leaves and oldest leaves in a shoot with high epiphyte density. A significant negative correlation was found between the epiphyte density and the photosynthetic activity of *Z. marina*, *Z. asiatica* and *P. iwatensis*, but no significant correlation was found in *Z. japonica* (Figure 3). This negative relationship between epiphytes and the photosynthetic activity was attributed to some factors such as aging and senescence, and material allocation strategy. Aging and senescence is an intrinsic factor and part of the physiological process in plants. But then, the decrease in the photosynthetic activity with leaf age was also considered as a strategy due to the effect of shading. Shading in seagrasses could either be as self-shading (within canopy) or direct shading by epiphytes. Self-shading within a seagrass canopy is less important due to the effect of water movement allowing the leaves to move freely. However, direct shading by epiphytes is more crucial to the photosynthetic activity of seagrasses. As a response against epiphytism, old leaves which are heavily colonized by the epiphytes would relocate its materials to the young growing leaves or shoots. In this manner, young leaves would have a higher photosynthetic activity compared to the old leaves. This strategy is not only a response against epiphytism, but also a good process to allow the survival of the whole plant.

Among the four temperate seagrass species, *P. iwatensis* showed the highest epiphyte density and it varied significantly with *Z. japonica*, *Z. marina* and *Z. asiatica* (Table 2). The highest epiphyte density in *P. iwatensis* was attributed to the presence of calcareous red algae (*P. zostericola*) which were abundant in the seagrass species. This observation is supported by Hamamoto et al (1996), finding the greatest coverage of red calcareous alga (*P. zostericola*) in the shoots of *P. iwatensis*.

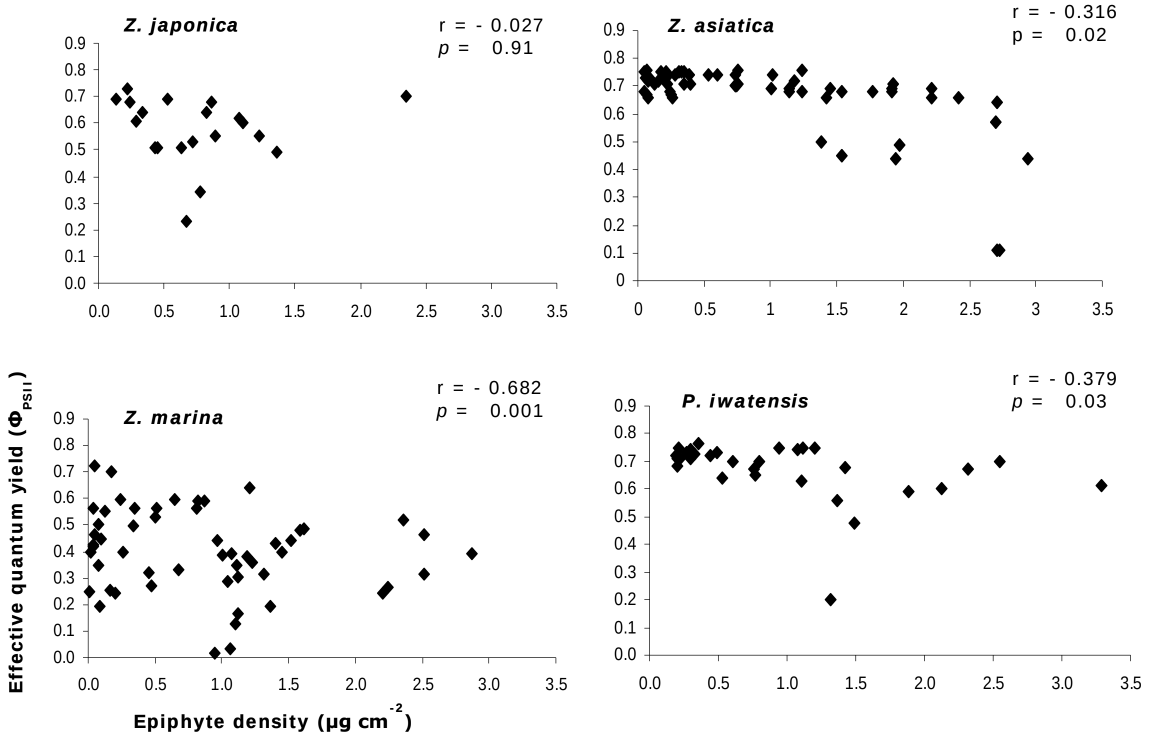

Figure 3. The relationship between epiphyte density and photosynthetic activity of the four temperate seagrass species (mean ± SE; n = 36).

Table 2

Comparison in the epiphyte density among species[a] of temperate seagrasses in Akkeshi Bay and Akkeshi-ko (mean ± SE, *n* = 36).

Species	*Epiphyte density (μg Chl a cm^{-2})*	*P*
Z. marina	1.08 ± 0.12^{b}	<0.001
Z. asiatica	1.01 ± 0.17^{b}	
P. iwatensis	2.57 ± 0.19^{a}	
Z. japonica	0.76 ± 0.11^{c}	

[a] Values are means ± SE, values with different letters are significantly different as determined by Tukey's multiple comparison test (P = 0.05).

Conclusions. The relationship between epiphytes and photosynthetic activity of temperate seagrasses (*Z. japonica*, *Z. marina*, *Z. asiatica* and *P. iwatensis*) was inversely proportional. The lower photosynthetic activity was found in the older region and older leaves in a shoot which had the higher epiphyte cover. The result was attributed to the effect of aging and senescence, but most importantly, as a strategy against epiphytism. Among the four temperate seagrass species, *P. iwatensis* showed the highest epiphyte density.

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