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Variation in biomass and species composition of epiphytic community on the different aged leaves of *Zostera marina* (Chlorophyta)

Yoko Niimura[1)]*, Hitoshi Tamaki[2)], Goro Yoshida[1)], Toshinobu Terawaki[3)], and Kazuo Iseki[4)]

[1)] *National Research Institute of Fisheries and Environment of Inland Sea,*
2-17-5 Maruishi, Hatsukaichi, Hiroshima 739-0452, JAPAN
[2)] *Ishinomaki Senshu University, 1, Shinmito, Minamisakai, Ishinomaki, Miyagi 986-8580, JAPAN*
[3)] *Fisheries Research Institute, Toyama Prefectural Agricultural,*
Forestry & Fisheries Research Center, Takatsuka 364, Namerikawa, Toyama 936-8536, JAPAN
[4)] *Graduate School of Biosphere Science, Hiroshima University,*
1-4-4 Kagamiyama, Higashi -Hiroshima, Hiroshima 739-8528, JAPAN
**present address: Oceanic Planning Corporation, 2-10-11 Hama, Minato, Nagoya,*
Aichi 455-0036, JAPAN e-mail : niimura@op-spirit.co.jp

Abstract The development of the epiphytic community on the leaves of *Zostera marina* was followed by comparing variations in epiphytic organic carbon, chlorophyll *a*, cell abundance, and species composition between the younger and older leaves. Biomass of epiphytic community on *Z. marina* depended on leaf age; particulate organic carbon and cell abundance on the older leaves were 21 and 192 times higher than those on the youngest leaves, respectively. The abundant taxa of epiphytic community were *Leptolyngbia* sp. (Cyanobacteria), *Cocconeis scutellum*, *Campylopyxis garkeana*, and Gomphonemataceae (Bacillariophyceae), and the change of species composition of epiphytic community was independent on the leaf age. Significant relation between CHL*a* and POC showed organic matter on the leaves was consisted of algal cells. The amount of epiphytic POC was well correlated with diatom cell abundance ($P<0.1$), but not with total cell abundance. The prevalence of diatoms instead of Cyanobacteria on the leaves was shown by both chemical and taxonomic analyses. Low POC/DW ratios of epiphytic community on the older leaves indicated that inorganic suspended particles were more likely to adhere.

Key words: epiphytic community, species composition, particulate organic carbon, succession, *Zostera marina*

INTRODUCTION

While epipelic diatoms on tidal flats have received much research attention (e.g., Hoagland, 1983 ; Stevensen and Glover, 1993 ; Cahoon, 1999 ; Wolfstein et al., 2000), few studies have been made on the epiphytic diatoms (e.g. McMillan, 1977 ; Penhale, 1977 ; Tsukidate and Takamori, 1978 ; Tanaka et al., 1984 ; Coleman and Burkholder, 1994). Some evidence suggest that epiphytic community are an important source of high quality food for grazing invertebrates (Medlin, 1980 ; Mukai, 1993 ; Moncreiff and Sullivan, 2001 ; Hoshika et al., 2006), although little has been done on quantitative and taxonomical studies of epiphytic diatoms.

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The processes of succession of epiphytic communities is useful for understanding the environmental conditions, such as light (Hansson, 1992), water quality (Eminson and Moss, 1980), differences in habitat type (e.g. Steinman and McIntire, 1986) and differences in substrate (Eminson and Moss, 1980; Hamilton and Duthie, 1984), because the development of the epiphytic community was effected by these environmental conditions. We examined the succession of epiphytic community on the different aged leaves in *Zostera marina* by using chemical and taxonomic analyses in this study.

The role of epiphytes in coastal ecosystems can be demonstrated by chemical analyses, as some researchers have done (Penhale, 1977 ; Mukai et al., 1979 ; Coleman and Burkholder, 1994). Quantitative analysis using pigments and organic carbon can describe how epiphytic community develop on leaves, which can then be used to evaluate their importance in seaweed ecosystems. The present study was part of an interdisciplinary investigation of the dynamics of epiphytic community on the eelgrass *Zostera marina*.

MATERIALS AND METHODS

Two shoots of *Zostera marina*, complete with roots (Fig. 1a, b), were obtained by diving on September 25, 2002, at 0.6 m depth at Ajina in the northern part of Hiroshima bay in the Seto Inland Sea. The microalgal mats of a few millimeters thickness on *Z. marina* leaves were studied by scraping all surfaces of each leaf with a knife and suspending them into filtered sea water. The lengths and widths of leaves were measured to estimate the area of the leaf (both sides). We assumed the different aged leaves represent epiphytic succession. Leaves were labeled in order of age from the growing inside of the shoot (youngest leaf) to the outermost shoot (fifth leaf) (Fig. 1c).

An aliquot of the above water sample (which contain epiphytic cells) was filtered through a 25mm glass fiber filter (Whatman GF/F) and chlorophyll *a* (CHL*a*) and pheopigment concentrations were determined by fluorometry (TURNER DESIGN, Model 10-AU) after extraction with N,N-dimethylfolmamide (Suzuki and Ishimaru, 1990).

Other aliquots of the water sample were also filtered through precombusted (500℃ , 2 hr) 25mm glass fiber filters (Whatman GF/F) to determine particulate organic carbon (POC), particulate organic nitrogen (PON), and dry weight (DW). These filter samples were then dried at 60℃ for 48 hr and DWs

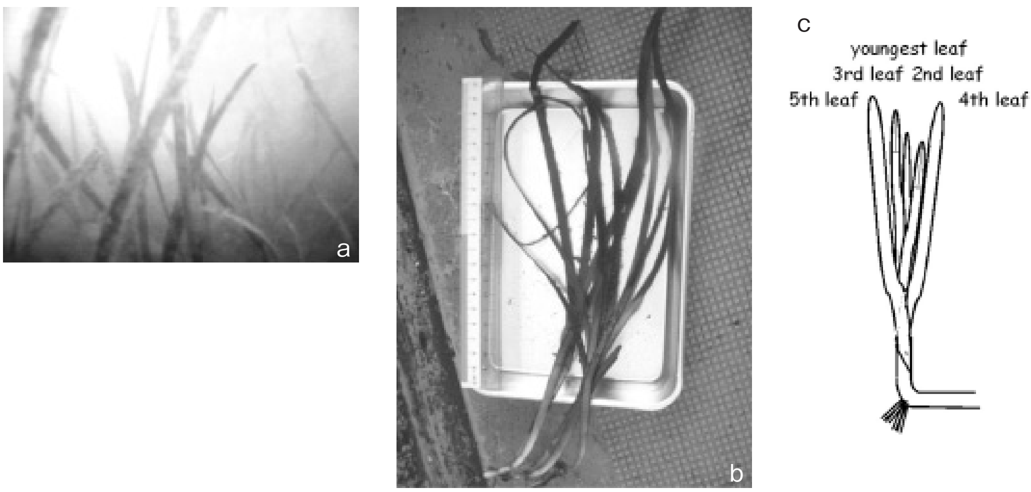

Fig. 1: Epiphytic community on *Zostera marina* in situ at the collection site at Hiroshima bay, Seto Inland Sea (a), after collection (b), and on the surface of leaves of different ages from youngest to oldest (5th) leaf (c).

were measured. Subsamples were then analyzed for POC and PON by an elemental analyzer (ANCA-MS, Europe Scientific).

Taxonomic identification and enumeration of algal species were carried out by light microscopy. Diatom cells were cleaned by an acid treatment to remove some of the organic material and cleaned samples were mounted in Pleurax (Von Stosch, 1974). A total of >400 cells were counted for each sample to avoid the influence of sample size on the relative abundances of species, unless it was not enough.

RESULTS AND DISCUSSION

1. Chemical analyses of epiphytic community on *Z. marina* leaf

The biomass of epiphytic community, as indicated by DW, CHL*a*, Pheopigments and PON, were undetectable on the youngest leaves but increased with leaf age from the second to the fourth leaf followed by a subsequent decrease on the fifth leaf (Fig. 2, 3, 4).

CHL*a* concentrations increased from 0.002 mgCHL*a* cm^{-2} on the second leaf to 0.011 mgCHL*a* cm^{-2} on the fourth leaf, followed by subsequent decrease (0.008 mgCHL*a* cm^{-2}) on the fifth leaf (Fig. 3). Pheopigments was also not detected on the 2nd leaf but steadily increased from 0.001mgPheo cm^{-2} on the third leaf to 0.003mgPheo cm^{-2} on the fifth leaf (Fig. 3). POC and PON showed a similar trend to the chlorophyll *a* concentrations (Fig. 4). These results indicate that the increasing biomass of epiphytic community depended on the age of *Z. marina leaves*.

The POC/DW ranged from 0.11 to 0.14, showing that older leaves had less organic material attached to their surfaces compared to the second leaf (Fig. 4). Epiphytic algal species may aggregate inorganic

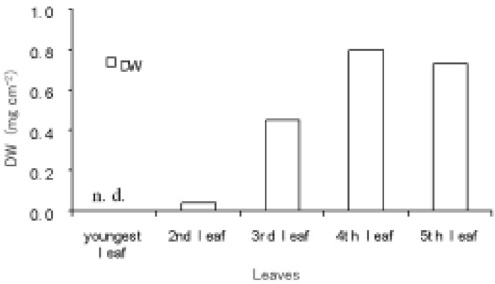

Fig. 2: Variance of dry weight (DW) of epiphytic community on the different aged leaves of *Zostera marina*. “n.d.” indicates “not detectable”.

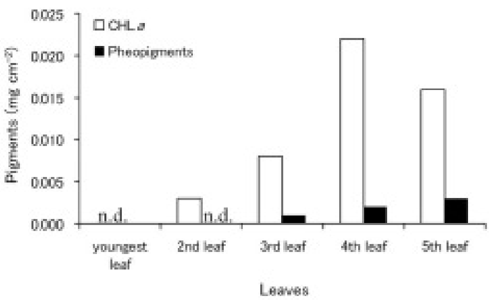

Fig. 3: Variance of chlorophyll *a* (CHL*a*) and pheopigments of epiphytic community on the different aged leaves of *Zostera marina*. “n. d.” indicates not detectable.

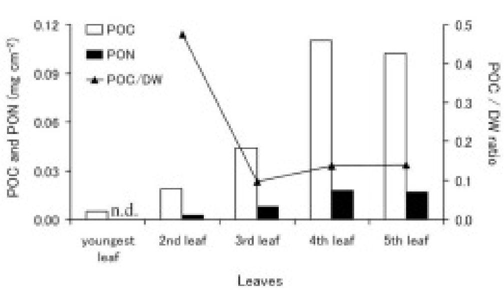

Fig. 4: Variance of particulate organic carbon (POC), particulate organic nitrogen (PON) and POC/DW ratio of epiphytic community on the different aged leaves of *Zostera marina*. “n. d.” indicates not detectable.

matters suspending in the water because microalgal and cyanobacterial species release a small amount of polysaccharidic material into the surrounding water and form mucilaginous aggregates (Phillippis et al. 2005).

The POC/PON ratios ranged from 5.6 to 6.3, and the POC/CHL*a* ratios ranged from 5.0 to 6.4. These pieces of evidence suggest that organic particles on the leaves are mostly the active growing epiphytic cells (Goldman et al., 1979) under high light irradiance / high temperature condition (Geider, 1987).

2. Microscopic analysis of epiphytic community on *Z. marina* leaves

The cell abundance of epiphytic community increased from 37 cells cm^{-2} on the youngest leaf to 10752 cells cm^{-2} on the third leaf (Fig. 5). The change of cell abundance was dependent on the leaf age. *Leptolyngbia* sp. (Cyanobacteria) and Gomphonemataceae, *Cocconeis scutellum* and *Campylopyxis garkeana* (Bacillariophyceae) were abundant in all different aged leaves (Table 1). The increasing POC coincides with the increase of diatom cell abundance ($P<0.1$), but not with total cell abundance (Fig. 6). Since cell volume of Cyanobacteria was about 1/100 to 1/700 times smaller, compared to abundant diatom taxa (Olenina et al., 2006), the contribution of Cyanobacteria to POC was considered to be small. The prevalence of diatom taxa in organic matter of epiphytic community on *Z. marina* leaves was shown by both chemical and taxonomic analyses.

The abundant diatom groups except *Cocconeis* and *Navicula* species produced three-dimensional communities on the leaves by forming threads and mucilage stalks. Such communities have been shown to develop on rocky substrates under low grazing pressure and favorable light conditions (Kawamura, 1994). Assuming the leaves of *Z. marina* were the same as rocky substratum, environmental conditions affecting epiphytes might be similar as Kawamura (1994) described when sampling was conducted.

This study has demonstrated that biomass of epiphytic community varies considerably depending on leaf age. For a more complete understanding of these processes, accurate knowledge of the seeding and the development of epiphytic community in the early stage is required.

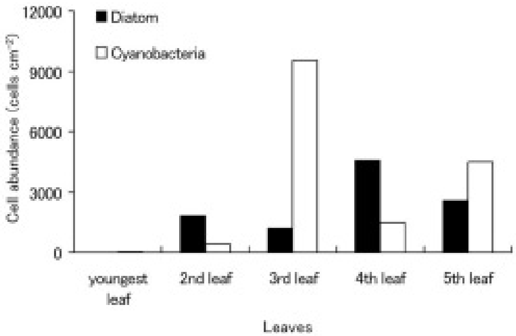

Fig. 5: Variance of diatom and cyanobacteria cell abundances on the different aged leaves of *Zostera marina*.

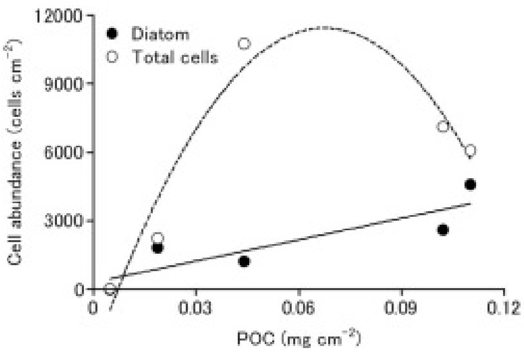

Fig. 6: Relations between POC and total and diatom cell abundances of epiphytic community on the different aged leaves of *Zostera marina*.

Table 1. Difference of attaching abundant species inhabiting on each leaf of *Z. marina*.

Leaves	Dominant sp. / gen.	Cell abundance (cells cm^{-2}) Relative abundance (%)	Secondly abundant taxa	Cell abundance (cells cm^{-2}) Relative abundance (%)	Thirdly abundant taxa	Cell abundance (cells cm^{-2}) Relative abundance (%)
youngest leaf	*Leptolyngbya* sp.	37 (100)	–		–	
2nd leaf	*Cocconeis scutellum*	1044 (57)	*Leptolyngbya* sp.	402 (18)	Gomphonemataceae	220 (12)
3rd leaf	*Leptolyngbya* sp.	9531 (47)	Gomphonemataceae	61 (5)	*Cocconeis scutellum*	55 (<5)
4th leaf	*Campylopyxis garkeana*	2026 (44)	*Leptolyngbya* sp.	1468 (24)	Gomphonemataceae	322 (7)
5th leaf	*Leptolyngbya* sp.	4508 (39)	Gomphonemataceae	782 (11)	*Navicula* sp.	711 (10)

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アマモの葉上着生群集の現存量および種組成の葉齢間における変動

新村陽子[1)]*・玉置　仁[2)]・吉田吾郎[1)]・寺脇利信[3)]・井関和夫[4)]

[1)] 水産総合研究センター瀬戸内海区水産研究所，〒739-0452 広島県廿日市市丸石2-17-5
[2)] 石巻専修大学，〒986-8580 宮城県石巻市南境新水戸1番地
[3)] 富山県農林水産総合技術センター水産研究所，〒936-8536 富山県滑川市364
[4)] 広島大学大学院生物圏科学研究科，〒739-8528 広島県東広島市鏡山1-4-4
*現住所：（株）海洋プランニング　〒455-0036 愛知県名古屋市港区浜2-10-11

要　約　沿岸生態系や物質循環において重要な役割を果たしていることが示唆されつつも，葉上着生群集の種の遷移や現存量の経時的な変化を調べた例は少ない。そこで，アマモ葉上に生育する着生群集を採取し，その現存量と種組成を葉ごとに調べ，葉齢間で比較した。その結果，現存量（クロロフィル*a*，粒状有機炭素・窒素）と細胞数は葉齢が高くなるほどに高密度であり，最も若い葉を基準にするとその差はPOCで最大21倍，細胞数で最大192倍であった。優占種はシアノバクテリアの*Leptolyngbya* sp.，珪藻類の*Cocconeis scutellum*, *Campylopyxis garkeana*, Gomphonemataceaeなどであったが，葉齢と優占種の変化には統計的に有意な差は認められなかった。着生群集中のPOCは珪藻類の細胞数と有意な相関が認められたが，全細胞数との間では認められなかった。このことはシアノバクテリアの*Leptolyngbya* sp. は細胞数で優占したが，細胞体積が珪藻に比べて顕著に小さい（100～700分の1程度）ためにPOCの増加への寄与が小さいことが原因と考えられた。すなわち，葉上着生群集の中で，珪藻類が有機炭素で示される現存量の主体であることが示唆された。さらに，葉齢の高いものはPOC/DWが低く，無機物含量の多い浮泥等がより付着しやすい環境になっていたと考えられる。

キーワード：葉上着生群集，珪藻類，遷移，炭素量，アマモ