

Species Composition of the Epiphytic Diatoms on the Leaf Tissues of Three *Zostera* Species Distributed on the Southern Coast of Korea

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The epiphytic diatom flora living on leaf tissues of *Zostera marina*, *Z. japonica* and *Z. caespitosa* at three study sites (Dadae, Gabae, and Jangmok) located in Geoje Island has been examined from July 2001 to March 2002 bimonthly. Total 81 species in 26 genera of diatoms have been observed on leaf tissues of the 3 *Zostera* species throughout the present study. *Cocconeis* spp. were predominant on *Z. japonica*, whereas *Navicula* spp. and *Nitzschia* spp. were predominant on *Z. marina* and *Z. caespitosa*. The community structure of the diatom assemblages varied according to the seagrass species. Species compositions of epiphytic diatoms appear to be closely related to morphology and lifespan of seagrasses.

Key Words: epiphytic diatom, *Zostera caespitosa*, *Z. japonica*, *Z. marina*, seagrass

INTRODUCTION

Epiphytes on seagrasses are sessile plants and animals that grow attached to their seagrass hosts. Epiphytic algal community on seagrasses is composed of variety species of diatoms, red, brown, green algae and cyanophyta (Frankovich and Zieman 1994). The epiphytic algal community is an important component in the seagrass meadow, which is one of the most productive coastal ecosystems. The production of algal epiphytes on *Zostera marina*, for example, can account for 40% of the total community production (Penhale 1977). Moncreiff *et al.* (1992) reported that the production of epiphytic algae often exceeded that of seagrass. It is obvious that diatoms are the most important structural elements of the epiphyton on seagrasses, because they take the greatest share in the epiphytic biomass (Jacobs and Noten 1980). In addition, the density of diatoms on seagrass was greater than on the neighboring rock wall. Epiphytic algal species were more frequently found in the fish guts than the algal species in the mud, suggesting that these epiphytic algae are important for the diet of fish and animals in coastal areas (Phillips and McRoy 1990). Although epiphytic diatom among epiphytic algae is an important component of seagrass

habitats, little research has been conducted on species composition of epiphytic diatoms on seagrass leaf tissues.

Davis (1913) reported 48 macroalgal species growing on *Zostera marina* in Massachusetts, USA. An extensive annotated list and key of algal epiphytes on *Thalassia testudinum* in Florida were compiled by Humm (1964). These algal epiphytes were mainly encrusting corallines and small brown algae. Ballantine and Humm (1975) also recorded 66 epiphytic algal species, which were composed of 30 red algae, 8 brown algae, 14 green algae, and 14 cyanophyta. Coleman and Burkeholder (1995) reported 15 epiphytic diatoms on *Zostera marina* in North Carolina. Despite many researches on epiphytic macroalgae community have been conducted in seagrass ecosystems, only few study has investigated the epiphytic diatoms. Furthermore, there are few researches on both epiphytic macroalgae and diatoms on seagrasses distributed on the coasts of Korea. Huh *et al.* (1998) only reported seasonal variation of epiphytic macroalge including red algae on *Zostera marina* in Kwangyang Bay on the southern coast of Korea. Many researches on freshwater or planktonic diatoms have been conducted (e.g. Lee and Chang 1996; Cho 2000; Cho 2001), however, there are few researches on epiphytic diatoms on seagrasses in Korea. In the present study, we examined the species composition of epiphytic diatoms on 3 *Zostera* species distributed on the southern coast of Korea. This

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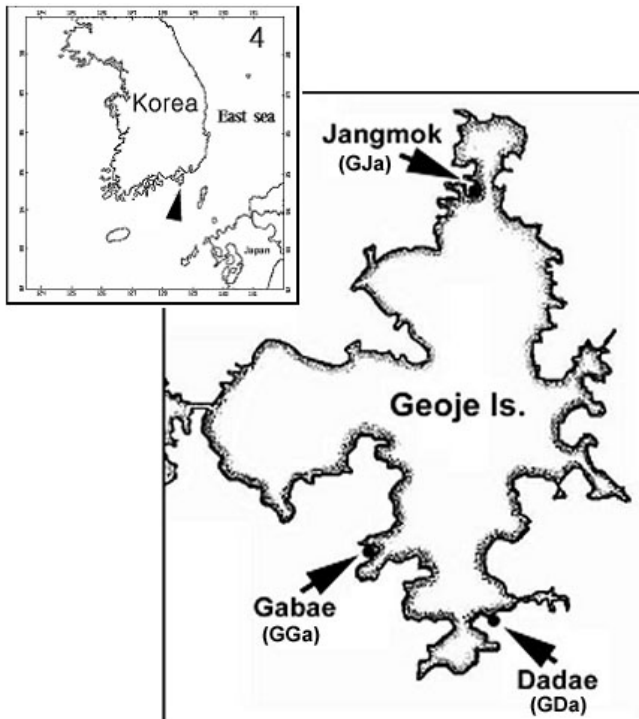


Fig. 1. Sampling sites in Geoje Island on the southern coast of Korea.

study is the first attempt to investigate epiphytic diatoms on seagrass species in Korea.

MATERIALS AND METHODS

Seagrasses and epiphytes sampling

Three seagrass species, *Zostera marina*, *Z. japonica* and *Z. caespitosa*, were collected from Dadae (GDa), Gabae (GGa), and Jangmok (GJa) sites within Geoje Island from July 2001 to March 2002 bimonthly (Fig. 1). Ten seagrass shoots with associated epiphytes were randomly collected during each sampling time. Shoots of *Z. japonica* were collected from the GDa site during low tide, while shoots of *Z. marina* were collected using SCUBA from the GDa, GGa, and GJa sites. *Z. caespitosa* was also sampled using SCUBA from the GJa site. The GDa and GGa sites were located in intertidal zone, but the GJa site was located in subtidal zone. *Z. japonica* is distributed in intertidal zone, whereas *Z. marina* is distributed in both intertidal and subtidal zones where the water depth was less than 5 m. *Z. marina* in the GDa and GGa sites was sometimes exposed to the air during the ebb tides. *Z. caespitosa* is distributed in deeper water (3–8 m) than *Z. marina*. Leaves and stems of seagrass were clipped above 1 cm from sediment surface to

minimize disturbance-related epiphytes loss and mixing with benthic diatoms and were preserved using neutralized formaldehyde (4%). Upon return to the laboratory, the shoots were gently rinsed with distilled water for removal of detritus and sediment. Epiphytes were separated from the seagrass leaves using the soft rubber stopper and brush.

Observation and identification of epiphytic diatoms

Epiphytes removed from seagrass were cleaned using the H_2O_2 method (Snoeijs 1994). The epiphytic diatoms without organic matter were rinsed with distilled water 10 times using centrifugation. Cleaned material for SEM was air-dried on glass cover slips naturally, coated with Au (3min, PARAONE ps-1200), and observed with a TOPCON-sm 300 operated 15Kv. Epiphytic diatoms on cover slip prepared using the air-dried method were more uniformly distributed than those using dry oven. For diatom identification, all diatom samples were observed with scanning electron microscopy (SEM). Species of diatoms were identified with the aid of works by Karsten (1928), Hustedt (1930), Silva (1962), and Round *et al.* (1990).

RESULTS AND DISCUSSIONS

Total 81 diatom species (2 orders, Centrales and Pennales; 3 suborders; 8 families; 26 genera) were observed throughout the experiment (Table 1). Approximately 50% of these taxa were obligate epiphytic diatoms, including species and varieties of the genera *Cocconeis*, *Achnanthes*, and *Tabularia* (McIntire and Moore 1977; Round *et al.* 1990). In addition, tube forming species of *Navicula* and species of *Navicula*, *Nitzschia* and *Amphora* that form masses of cells within a gelatinous matrix can also be considered as real epiphytes (Main and McIntire 1974; Round *et al.* 1990). At all study sites, diatom species of *Berkaleya lutilans*, *Cocconeis heteroidea*, *C. placentula*, *C. scutellum*, *Fragilaria hyalina*, *Gomphonemopsis exigua*, *Grammatophora oceanica*, *Hantzschia spectabilis*, *Haslea ostrearia*, *Navicula perminuta*, *Nitzschia sigmoidea*, *N. palea* were more frequently observed (Fig. 2). *Skeletonema costatum*, which belongs to Order Centrales, was observed at only one site (GDa). It is widely known that *S. costatum* is planktonic species (Shim 1994). Therefore, it can be suggested that *S. costatum* was attached to seagrass temporarily during blooming season.

On *Zostera marina* leaf tissues, 33 species (18 genera) of

Table 1. Epiphytic diatoms on leaf tissues of seagrasses from Geoje Island on the southern coast of Korea.

Species	Sites	GGa		GDa		GJa	
		<i>Z. marina</i>	<i>Z. marina</i>	<i>Z. japonica</i>	<i>Z. marina</i>	<i>Z. caespitosa</i>	
Order Pennales							
Suborder Araphidineae							
Family Diatomaceae							
<i>Delphineis surirella</i> (Ehrenberg) G. Andrews 1981			+	+		+	+
<i>Diatoma elongatum</i> Agardh 1824					+		
<i>Fragilaria hyalina</i> (Kützing) Grunow 1862		+	+	+		+	+
<i>F. elliptica</i> Schumann 1858		+	+			+	+
<i>Grammatophora marina</i> (Lyngbye) Kützing 1844		+	+			+	+
<i>G. oceanica</i> (Ehrenberg) Grunow 1881		+	+	+		+	+
<i>Rhabdonema adriaticum</i> Kützing 1844			+			+	+
<i>Tabularia barbata</i> (Kützing) Williams 1986			+			+	
<i>T. fasciculata</i> (Agardh) Williams 1986		+	+	+		+	+
<i>T. parva</i> (Kützing) Williams 1986		+	+			+	
<i>Bacillaria paxillifer</i> (Müller) Hendey 1951		+	+			+	
Suborder Raphidineae							
Family Achnantheaceae							
<i>Achnanthes brevipes</i> Agardh 1824			+	+		+	+
<i>A. brevipes</i> var. <i>angustata</i> (Grev.) Cleve 1895			+				
<i>A. delicatula</i> Grunow 1880			+				
<i>A. longipes</i> Agardh 1824		+	+			+	+
<i>A. pusilla</i> Grunow 1880		+	+				+
<i>A. taeniata</i>			+				
<i>A. sp. 1</i>						+	
<i>A. sp. 2</i>			+				+
Family Cocconeaceae							
<i>Cocconeis britannica</i> Ehrenberg 1838			+			+	
<i>C. heteroidea</i> Hantzsch 1863		+	+	+		+	+
<i>C. maxima</i> Peragalla & Peragallo 1897		+	+	+			+
<i>C. neothumensis</i> var. <i>marina</i> Stefano 2000				+			
<i>C. placentula</i> Ehrengerg 1838		+	+	+		+	+
<i>C. pseudomarginata</i> Gregory 1855			+	+		+	+
<i>C. scutellum</i> Ehrengerg 1838		+	+	+		+	+
<i>C. scutellum</i> var. <i>posidoniae</i> Stefano 2000				+			
<i>C. stauroneiformis</i> (Ranenhorst) Okuno 1957			+	+			+
<i>C. sp. 1</i>			+				
<i>C. sp. 2</i>			+				
Family Eunotiaceae							
<i>Eunotia</i> sp.			+				
Family Cymbellaceae							
<i>Amphora libyca</i> Ehrenberg 1840			+			+	
<i>A. pediculus</i> (Kützing) Grunow 1880			+				
<i>A. ovalis</i> Kützing 1844		+	+			+	+
<i>A. terroris</i> Ehrenberg 1853			+				+
<i>A. sp. 1</i>		+	+				
<i>A. sp. 2</i>			+				+
<i>A. sp. 3</i>			+				
<i>Cymbella brehmii</i> Hustedt 1912			+	+			
<i>C. gracilis</i> (Ehrenberg) Kützing 1844			+				+
<i>C. lanceolata</i> (Ehrenberg) Van Heurck		+	+				
<i>C. sp. 1</i>			+			+	
<i>C. sp. 2</i>			+				

Table 1. (continued)

Species	Sites	GGa		GDa		GJa	
		<i>Z. marina</i>	<i>Z. marina</i>	<i>Z. japonica</i>	<i>Z. marina</i>	<i>Z. caespitosa</i>	
<i>C. sp. 3</i>			+				
<i>C. sp. 4</i>			+				
<i>Diploneis crabo</i> (Ehrenberg) Ehrenberg ex Cleve 1894		+	+		+		
<i>D. ovalis</i> (Hilse) Cleve 1894			+				
<i>Gomphonemopsis exigua</i> (Kützing) Medlin 1986		+	+	+	+		+
Family Naviculaceae							
<i>Amphipleura pellucida</i> Kützing 1844		+	+				
<i>Anomoeoneis</i> sp.			+				
<i>Berkaleya rutilans</i> (Trent.) Grunow 1868		+	+	+	+		+
<i>Haslea ostrearia</i> Grunow 1868		+	+	+	+		+
<i>Mastogloia pusilla</i> Grunow 1878					+		
<i>M. smithii</i> Thwaites 1856		+	+		+		
<i>Navicula gregaria</i> Dolkin 1861			+				+
<i>N. perminuta</i> Grunow in Van Heurck 1880		+	+	+	+		+
<i>N. radiosa</i> Kützing		+	+	+			
<i>N. ramosissima</i> (Agardh) Cleve 1895		+					+
<i>N. spectabilis</i> Gregory 1855		+	+				
<i>N. sp. 1</i>				+			
<i>N. sp. 2</i>			+				
<i>N. sp. 3</i>			+		+		
<i>Rhoicosphenia abbreviata</i> (Agardh) Lange-bertalot 1980			+				
Family Nitzschiaceae							
<i>Cylindrotheca gracilis</i> Grunow 1880			+				+
<i>Denticula</i> sp.							+
<i>Hantzschia spectabilis</i> (Ehrenberg) Hustedt 1959		+	+	+	+		+
<i>Nitzschia bifucata</i> Cleve 1900			+				
<i>N. constricta</i> Grunow 1880		+	+		+		+
<i>N. dissipata</i> (Kützing) Grunow 1862		+	+		+		+
<i>N. filiformis</i> (Wm. Smith) Van Heurck 1896			+	+			
<i>N. frustulum</i> (Kützing) Grunow 1880			+		+		
<i>N. gracilis</i> Grunow 1884			+		+		
<i>N. laevis</i> Grunow in Cleve et Müller 1882			+	+			
<i>N. microcephala</i> Grunow in Cleve et Grunow 1880			+				
<i>N. obtusa</i> var. <i>scalpelliformis</i> Grunow in van Heurck 1881					+		+
<i>N. paleacea</i> Grunow 1884		+	+	+	+		+
<i>N. panduriformis</i> Gregory 1857		+	+				
<i>N. sigmoidea</i> W. Smith 1853		+	+	+	+		+
<i>N. sp. 1</i>			+				
<i>N. sp. 2</i>			+		+		
<i>N. sp. 3</i>		+	+				
Order Centralis							
Suborder Coscinodiscaneae							
Family Skeletonemaceae							
<i>Skeletonema costatum</i> (Greville) Cleve 1873			+				

epiphytic diatoms in the GGa site, 73 species (24 genera) in the GDa site, and 38 species (17 genera) in the GJa site were observed during the experiment. On *Z. japonica*, 26 diatom species (14 genera) were found in the GDa site, while the epiphytic diatoms of 35 species (17 genera)

were observed on *Z. caespitosa* in the GJa site. Most of the diatoms were found from at least 2 seagrass species. However, *Diatoma elongatum*, *Cocconeis neothumensis* var. *marina*, *C. scutellum* var. *posidoniae*, and *Navicula* spp. (4 species) were observed only on *Z. japonica*. *Cocconeis*

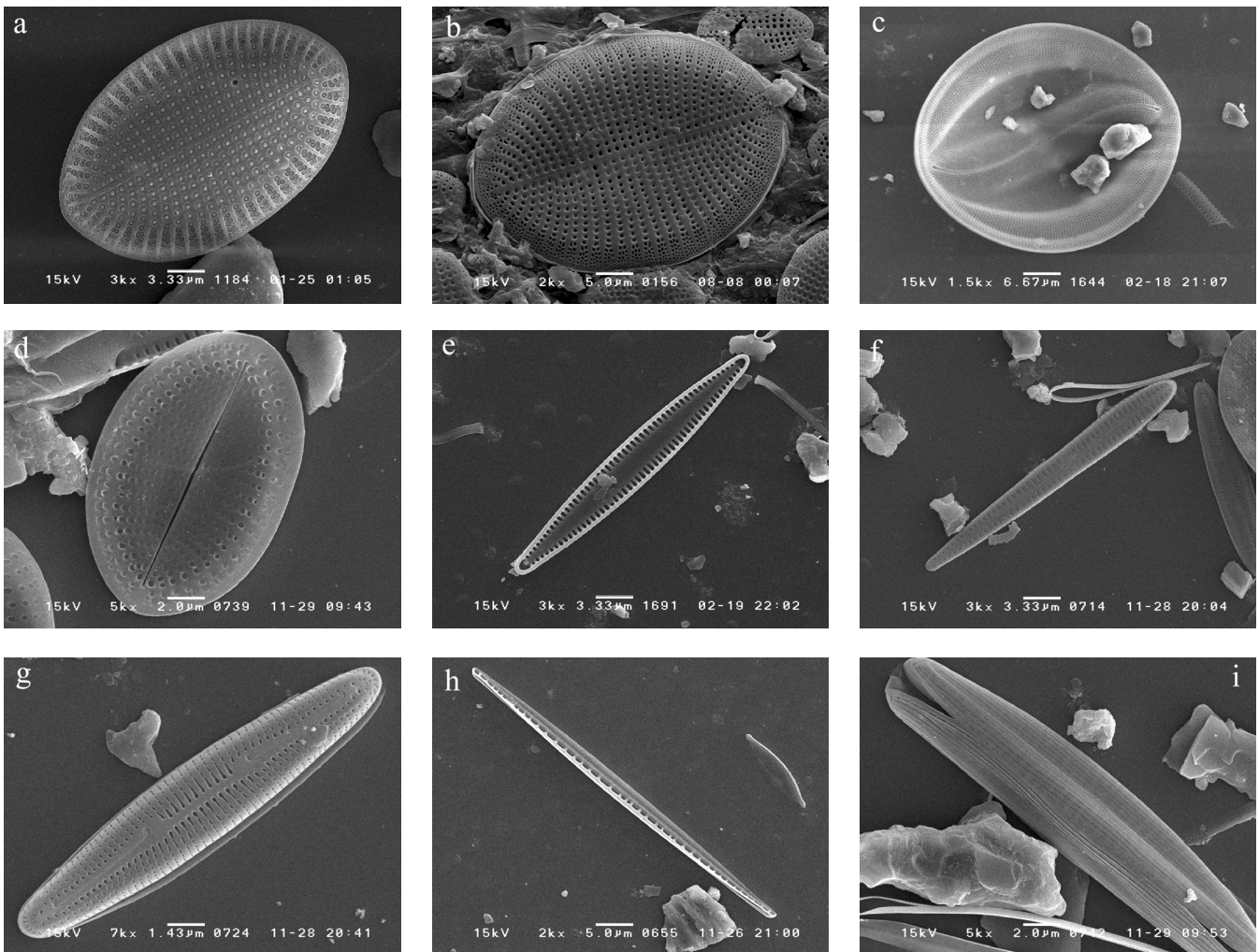


Fig. 2. SEM photos of epiphytic diatoms on seagrass leaf surface. a, b: *Cocconeis scutellum*; c: *C. heteroidea*; d: *C. placentula*; e: *Tabularia fasciculata*; f: *Gomphonemopsis exigua*; g: *Berkeleya rutilans*; h: *Nitzschia sigmoidea*, i: *Haslea ostrearia*

placentula and *C. scutellum* were dominant species and accounted for over 40% of total frustule number on *Z. japonica* at GDa site for study period except November, 2002 (*Berkeleya rutilans*, 31.6%; *C. scutellum*, 24.6%). *Tabularia fasciculata* (Jul. 2001, 31.6%), *Gomphonemopsis exigua* (Sep. 2001, 48.6%), *Berkeleya rutilans* (Nov. 2001, 59.0%; Jan. 2002, 32.1%) and *Nitzschia sigmoidea* (Mar. 2002, 36.8%) were dominant species on *Z. marina* at GDa sites. Generally, reasons for a specific dependence of substratum of the epiphytic diatoms may be the shape of the thalli surface (Ramm 1976). On fine-branched thalli surface, diatoms with a small attaching area such as *Licmophora* and *Synedra* are predominant. Thick-branched foliose thalli are preferred by diatom species with a large attaching area (*Cocconeis*), tube-living diatoms, and *Rhoicosphenia curvata* (Ramm 1976). In this study, dominant epiphytic diatoms on *Z. marina*, which has thick and wide leaves, were tube living diatoms

(*Navicula* sp.) like as the result of Ramm (1976). In contrast, oval forms with a large attaching area such as *Cocconeis* spp. were predominant on *Z. japonica* which has thin and narrow leaves. The comparison of diatom genera (*Cocconeis*:*Navicula*:*Nitzschia*) was clearly showed differences in diatom species composition on different seagrass species. The ratio of the diatom genera were 9:6:14 on *Z. marina* and 8:3:4 on *Z. japonica* in the GDa site, while the ratios were 5:2:8 on *Z. marina* and 6:3:5 on *Z. caespitosa* in the GJa site.

The following colonization sequence was defined from the study of fouling in freshwater system by Hoagland *et al.* (1982): 1. formation of an organic film, 2. settlement by bacteria, 3. settlement by opportunistic diatoms, 4. formation of a more complex diatom community at the base of the diatom crust. However, diatoms and bacteria arrived at the same time during the initial epiphyte colonization process on *Zostera marina* leaf surface and

organic film formation and bacteria settlement prior to diatom settlement were not observed (Jewett-Smith 1989). Additionally, the diatom *C. scutellum* was the primary colonizer on the newly emergent blade surfaces of *Z. marina* (Sieburth and Thomas 1973). These reports suggested that species composition of the epiphytic toms can be affected by the lifespan of seagrass leaves. Generally, lifespan of *Z. marina* leaf tissues is about twice as long as *Z. japonica*. The leaf lifespan of *Z. marina* averaged 48 day along the low intertidal area and 36 day along the high intertidal area (Kentula and McIntire 1986). However, leaf lifespan of *Z. japonica* averaged 21-22 days (Lee *et al.* 2005). Early colonizers such as the genus *Cocconeis* (Sieburth and Thomas 1973) were easily observed on *Z. japonica*, but the late colonizing diatoms such as *Navicula* and *Nitzschia* appear to be not easily established on *Z. japonica* which has shorter leaf lifespan than *Z. marina*.

This study demonstrated species composition of the epiphytic diatoms on 3 seagrass species distributed on the coast of Korea and effects of lifespan and surface structure of seagrass leaf tissues on the species composition of the epiphytic diatoms. However, the species composition should be changed by various environmental factors and season as well as lifespan and surface structure of seagrass leaf. Therefore, seasonal dynamics of epiphytic diatoms and effects of various environmental factors on epiphytic diatom growth should be examined in the future study.

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REFERENCES

- Ballantine D.L. and Humm H.J. 1975. Benthic algae of the Anclote Estuary. I. Epiphytes of seagrass leaves. *Florida Sci.* **38**: 144-149.
- Cho K.J. 2000. Epiphytic Diatom Flora of Lakes around Kumho District of North Korea. *Algae* **15**: 255-286.
- Cho K.J. 2001. Planktonic and Periphytic Diatom Flora of the Freshwaters of the Kumho District and Its Vicinity in North Korea. *Algae* **16**: 75-84.
- Coleman V.L. and Burkholder J.M. 1995. Response of microalgal epiphyte communities to nitrate enrichment in an eelgrass (*Zostera marina*) meadow. *J. Phycol.* **31**: 36-43.
- Davis B.M. 1913. General characteristics of the algal vegetation of Buzzards Bay and Vineyard Sound in the vicinity of Woods Hole. *Bull. Bur. Fish.* **31**: 443-544.
- Frankovich T.A. and Zieman J.C. 1994. Total epiphyte and epiphytic carbonate production on *Thalassia testudinum* across Florida Bay. *Bull. Mar. Sci.* **54**: 679-695.
- Hoagland K.D., Romer S.C. and Rosowski J.R. 1982. Colonization and community structure of two periphon assemblages, with emphasis on the diatoms (Bacillariophyceae). *Amer. J. Bot.* **69**: 188-213.
- Huh S-H, Kwak S.N. and Na K.W. 1998. Seasonal Variations of Eelgrass (*Zostera marina*) and Epiphytic Algae in Eelgrass Beds in Kwangyang Bay. *J. Korean Fish. Soc.* **31**: 56-62.
- Humm H.J. 1964. Epiphytes of the seagrass, *Thalassia testudinum*, in Florida. *Bull. Mar. Sci. Gulf Carib.* **14**: 306-341.
- Hustedt F. 1930. Die Kieselalgen Deutschlands. In: Rabenhorst L. (ed.), *Kryptogamen-Flora*. Leipzig, Germany. pp. 1-920.
- Jacobs R.P.W.M. and Noten T.M.P.A. 1980. The annual pattern of the diatoms in the epiphyton of eelgrass (*Zostera marina* L.) at Roscoff, France. *Aquat. Bot.* **8**: 355-370.
- Jewett-Smith J. 1989. *The seagrass and its epiphytic diatoms: A field and laboratory investigation*. Ph. D. Thesis. The University of Texas at Austin, USA, 188 pp.
- Karsten G. 1928. Bacillariophyta (Diatomeae). In: Engler and Pranti (eds), *Die Natürlichen Pflanzenfamilien: Peridineae, Diatomae, Myxomycetes*. Leipzig, Germany. pp. 105-303.
- Kentula M.E. and McIntire C.D. 1986. The autecology and production dynamic of eelgrass (*Zostera marina* L.) in Tetarts Bay, Oregon. *Estuaries* **9**: 188-199.
- Lee J.H. and Chang M. 1996. Morphological Variations of the Marine Diatom Genus *Actinoptychus* in the Coastal Waters of Korea. *Algae* **11**: 365-374.
- Lee S.Y., Oh J.H., Choi C.I., Suh Y. and Mukai H. 2005. Leaf growth and population dynamics of intertidal *Zostera japonica* on the western coast of Korea. *Aquat. Bot.* **83**: 263-280.
- Main S.P. and McIntire C.D. 1974. The distribution of epiphytic diatoms in Yaquina Estuary, Oregon (USA). *Bot. Mar.* **17**: 88-99.
- McIntire C.D. and Moore W.W. 1977. Marine littoral diatoms: ecological considerations. In: Werner D. (ed.), *The Biology of Diatoms*. Blackwell, Oxford. pp. 333-371.
- Moncreiff C.A., Sullivan M. J. and Daehnick A.E. 1992. Primary production dynamics in seagrass beds of Mississippi sound: The contributions of seagrass, epiphytic algae, sand microflora, and phytoplankton. *Mar. Ecol. Prog. Ser.* **87**: 161-171.
- Penhale P.A. 1977. Macrophyte-epiphyte biomass and productivity in an eelgrass (*Zostera marina* L.) community. *J. Exp. Mar. Bio. Ecol.* **26**: 211-224.
- Phillips C. and McRoy C.P. 1990. Seagrass research method. UNESCO, 210 pp.
- Ramm G. 1976. Structure of epiphytic diatom populations of the phytal of the Kiel Bight (Western Baltic). In: Simonsen R. (ed.), *Proceedings of Fourth Symposium on Recent and Fossil Marine Diatoms*. Ganter Verlag KG, Germany. pp. 379-387.
- Round F.E., Crawford R.M. and Mann D.G. 1990. *The diatoms: biology & morphology of the genera*. Cambridge University press, Cambridge, 747 pp.

- Shim J.H. 1994. *Illustrated encyclopedia of fauna & flora of Korea*, Vol. 34, *Marine Phytoplankton*. Ministry of Education, Korea, 487 pp.
- Sieburth J.McN. and Thomas C.D. 1973. Fouling on eelgrass (*Zostera marina* L.). *J. Phycol.* **9**: 46-50.
- Silva P.C. 1962. Classification of algae. Appendix A. In: Lewin R.A. (ed.), *Physiology and Biochemistry of Algae*. Academic Press, New York. pp. 827-837.
- Snoeijs P. 1994. Distribution of epiphytic diatom species composition, diversity and biomass on different macroalgal hosts along seasonal and salinity gradients in the Baltic sea. *Diatom Res.* **9**: 189-211.
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