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SEASONAL ABUNDANCE OF EPIPHYTIC DINOFLAGELLATES AROUND COASTAL WATERS OF JEJU ISLAND, KOREA

Md. Mahfuzur Rahman Shah¹, So-Jeong An², and Joon-Baek Lee²

Key words: seasonal abundance, epiphytic dinoflagellates, climate change, Jeju Island.

were less preferred host by most of the dinoflagellates.

ABSTRACT

The seasonal abundance of epiphytic dinoflagellates in the intertidal zone of Jeju Island, Korea was quantitatively estimated by monthly collection of macroalgal samples (Rhodophyta, Phaeophyta, and Chlorophyta) from six sampling locations from July 2012 to June 2013. Ten epiphytic dinoflagellate taxa, including eight potentially toxic species Amphidinium carterae, A. operculatum, Gambierdiscus sp., Ostreopsis ovata, Prorocentrum concavum, P. emarginatum, P. lima, and P. rhathymum, were identified. Two Amphidinium and three Prorocentrum species are newly recorded in Korean coastal waters. A significant change in seasonal abundance was recorded with maximum (751.82 \pm 223.12 cells g⁻¹ wet weight of algae; cells g⁻¹ hereafter) in June (summer), followed by October (autumn) (650.45 \pm 225.02 cells g⁻¹) and September (598.02 \pm 197.82 cells g⁻¹). O. ovata was the most abundant (338.21 \pm 11 cells g⁻¹), reported in October and Gambierdiscus sp. was the least abundant, which was found only in September $(6.92 \pm 16.97 \text{ cells g}^{-1})$ and October $(6.54 \pm$ 6.54 cells g⁻¹) at Hamduk (St 2). Significantly, highest spatial abundance of total dinoflagellates for all sampling stations was found at Hamduk (St 2) (547.91 \pm 315 cells g⁻¹), while it was lowest at Hwasun (St 5) (232.59 \pm 144.93 cells g⁻¹). Abundance of all dinoflagellate species was significantly correlated with environmental parameters, with some exceptions. During summer and autumn, increasing abundance of dinoflagellate at all sampling stations compared to the other seasons emphasizes environmental and biological interactions of epiphytic dinoflagellate with host macroalgae. Generally, each of the epiphytic dinoflagellates did not show specific preference of macroalgae as host. However, Chlorophytes

I. INTRODUCTION

Species in the genera Gambierdiscus, Ostreopsis, Coolia, Prorocentrum, and Amphidinium are known to be epiphytic and/or benthic dinoflagellates [25, 48]. Most of the epiphytic dinoflagellates in these genera are known to be potentially toxic [13, 36] and harmful to human as well as to marine organisms, alerting scientists, aquaculture industry, and government [35]. The most well known human intoxication because of benthic epiphytic dinoflagellates is ciguatera fish poisoning [19]. Globally, one million people may be affected by ciguatera annually [22], with estimated economic impact of ciguatera in the United States to be \$21.19 million per year on average [4]. The genus Gambierdiscus is the main causative agent of ciguatera. In particular, Prorocentrum lima, P. concavum, Ostreopsis siamensis, and O. ovata have been implicated in ciguatera fish poisoning based on distribution, toxicity to mice and the presence of a fat soluble toxic fraction [39, 51]. These organisms from epiphytic communities associated with coral reefs, or rather with macroalgae attached to coral surfaces. These assemblages may vary in species composition and cell concentration between sites [49]. The mixed association of toxic dinoflagellates may contribute to the polymorphism of the clinical features of ciguatera [52].

The majority of the benthic epiphytic dinoflagellates are reported from tropical or subtropical regions of the Pacific Ocean, Indian Ocean, and the Caribbean, and found associated with seagrasses, green, brown, and red algae, as well as dead coral and sediment [2, 18]. However, some species also live in temperate regions [44]. The occurrence of epiphytic and benthic dinoflagellates in temperate waters has been reported as evidence of increasing water temperature [26, 27, 43]. Seasonal/annual and interannual dynamics of benthic and epiphytic dinoflagellate assemblages in reef zone have been studied in the Virgin Islands [8], on Singapore reefs [24], and in the NW Mediterranean [50].

Jeju Island belonging to the temperate region classified based on air temperature and coastline is mainly composed of rocky shore and sandy beaches with a few sand tidal flats.

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	Table 1. Summary of sampling stations in the intertidal zone along the coasts of Jeju Island, Korea.									
Station Locations Latitude/ Longitude Characteristics of sampling stations										
1	SEHWA	33°31'29.86"N/126°51'40.50"E	Sandy beach with fine white sand and volcanic rocks							
2	HAMDUK	33°32'32.94"N/126°40'12.27"E	White sandy beach with fine white sand							
3	HYUPJAE	33°23'38.88"N/126°14'23.02"E	Large sandy beach with volcanic rocks							
4	HAMO	33°12'39.86"N/126°15'38.23"E	Very small beach with coarse black and white sand with volcanic rocks							

33°14'22.38"N/126°19'55.67"E

33°31'29.86"N/126°51'40.50"E

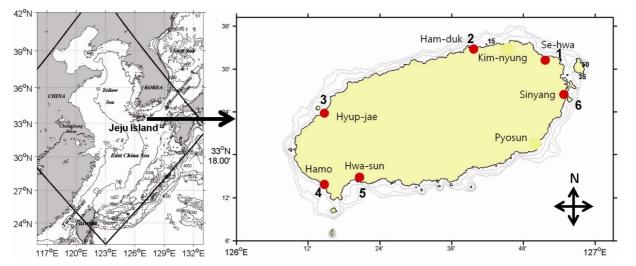


Fig. 1. Map showing Jeju Island, Korea and locations of six sampling stations along the coasts of Jeju Island.

Previously, the dinoflagellates recorded from this Island were planktonic but potentially toxic benthic and epiphytic species have not been well documented. Information on existing diversity and distribution patterns and abundance of epiphytic dinoflagellates around Jeju Island is limited. To date, no toxic event caused by a marine benthic dinoflagellate has been reported from Jeju Island. Kim et al. [29] reported abundance of epiphytic dinoflagellate in autumn 2009. Jeong et al. [26, 27] and Lim et al. [33] reported the presence of benthic epiphytic dinoflagellates in the coastal waters of Jeju Island. However, in-depth information on diversity and seasonal abundance of benthic epiphytic dinoflagellates from coastal waters of Jeju Island is needed.

5

6

HWASUN

SINYANG

The aim of this study was to survey epiphytic dinoflagellate community present in intertidal zone of the coastal waters of Jeju Island. The objectives of the this study were: (1) to determine the species composition of epiphytic dinoflagellates, (2) to evaluate seasonal changes of benthic and epiphytic dinoflagellates, (3) to relate the presence and abundance of dinoflagellates from various macroalgal substrates, (4) to determine the physical-chemical variables and their relation to the abundance of dinoflagellates. The results of this study can be used to ascertain the potential threat that toxigenic epiphytic dinoflagellates pose to coastal food webs and human health.

II. MATERIALS AND METHODS

Small beach with coarse black and white sand with volcanic rocks

Sandy beach with fine white sand and volcanic rocks

1. Study Sites and Sample Collection

This study was carried out from July 2012 to June 2013 in the intertidal zone along the coasts of Jeju Island, Korea. Monthly sampling was carried out at six stations (beaches) (Fig. 1). Total 247 samples of macroalgae including 30 macroalgal species (Rhodophyta, Phaeophyta, and Chlorophyta) were collected during the lowest low tide. A brief description of the characteristics, latitudes, and longitudes of the sampling stations is presented in Table 1. Hands picked macroalgae of ca. 20-100 g wet weight was placed into individual plastic Ziploc bags with ambient seawater. Samples were stored on ice cooler for transportation back to the laboratory.

In laboratory, each macroalgae was put in a plastic container with 200 mL of fresh filtered seawater were vigorously shaken to dislodge epiphytic dinoflagellate cells for 1 minute, and the material was passed through 200 and 100 µm mesh sieves to remove large particles and finally passed through a 20 µm mesh sieve. The material retained by the sieve was resuspended in sterile filtered seawater (25 mL) and fixed in 3.7% (final concentration) paraformaldehyde in filtered seawater. Wet weight of each macroalgal sample was determined using weighing balance. For quantitative analysis, 1 mL of preserved sample was counted triplicate using

Dipoflagallatas	2012						
Dinoflagellates	Jul	Aug	Sep	Oct	Nov	Dec	
Amphidinium carterae*#			20.63 ± 17.21^{b}	33.61 ± 26.61^{ab}	20.78 ± 14.72^{b}	12.03 ± 2.40^{b}	
A. operculatum*#	19.56 ± 21.33^{b}	16.60 ± 5.91^{b}	39.64 ± 20.64^{b}	53.71 ± 29.25^{ab}	24.13 ± 15.43^{b}	19.58 ± 9.86^{b}	
Coolia malayensis	48.52 ± 14.69^{b}	46.15 ± 17.66^{ab}	109.95 ± 43.82^{a}	104.85 ± 47.45^{a}	66.38 ± 48.13^{ab}	$52.65\pm9.27^{\rm b}$	
Gambierdiscus sp.*	0 ± 0	0 ± 0	6.92 ± 16.97^{a}	6.54 ± 16.04^{a}	0 ± 0	0 ± 0	
Ostreopsis ovata*	231.07 ± 87.87^{ab}	$160.69 \pm 81.69^{\mathrm{b}}$	311.03 ± 126.14^{a}	338.21 ± 119.54^{a}	196.61 ± 109.57^{a}	77.48 ± 46.43^{b}	
Prorocentrum concavum*#	30.12 ± 18.05^{ab}	12.79 ± 11.62^{b}	38.51 ± 25.60^{ab}	28.78 ± 15.64^{ab}	17.69 ± 20.45^{ab}	17.24 ± 15.22^{ab}	
P. emarginatum*#	6.40 ± 8.23^{a}	3.36 ± 4.30^{a}	9.75 ± 16.18^{a}	13.16 ± 17.98^{a}	2.77 ± 3.91^{a}	7.96 ± 13.18^{a}	
P. fukuyoi#	4.37 ± 4.93^{a}	7.62 ± 7.23^{a}	17.93 ± 19.48^{a}	23.23 ± 15.24^{a}	16.42 ± 16.63^{a}	14.19 ± 12.10^{a}	
P. lima*	12.71 ± 16.06^{a}	7.82 ± 11.21^{a}	24.09 ± 33.57^{a}	39.48 ± 48.91^{a}	8.76 ± 10.25^{a}	4.19 ± 6.67^{a}	
P. rhathymum*	13.70 ± 22.55^{a}	12.56 ± 14.31^{a}	14.99 ± 14.74^{a}	10.36 ± 18.01^{a}	11.07 ± 15.02^{a}	$9.7 \pm 9.53^{\mathrm{a}}$	
Total dinoflagellates	414.96 ± 137.86^{b}	273.48 ± 103.76^{bc}	$^{\circ}$ 598.02 ± 197.82 ^b	650.45 ± 225.02^{ab}	383.63 ± 153.75^{bc}	216.82 ± 81.67^{bc}	
Dipoflagallatas	2013						
Dinoflagellates	Jan	Feb	Mar	Apr	May	Jun	
Amphidinium carterae*#	14.82 ± 3.99^{b}	16.77 ± 8.34^{ab}	19.78 ± 18.97^{b}	22.47 ± 14.32^{ab}	36.75 ± 10.47^{ab}	76.39 ± 37.21^{a}	
A. operculatum*#	22.32 ± 7.13^{b}	27.45 ± 9.66^{b}	38.33 ± 9.52^{b}	35.28 ± 11.12^{b}	52.04 ± 17.50^{ab}	103.04 ± 37.60^{a}	
Coolia malayensis	23.03 ± 10.42^{b}	35.34 ± 10.33^{b}	59.39 ± 21.12^{ab}	64.90 ± 28.57^{ab}	81.02 ± 19.09^{ab}	131.31 ± 77.43^{a}	
Gambierdiscus sp.*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
Ostreopsis ovata*	$43.33 \pm 20.33^{\circ}$	60.91 ± 18.55^{bc}	95.71 ± 29.24^{bc}	123.52 ± 31.01^{ab}	172.79 ± 59.14^{b}	303.10 ± 64.83^{a}	
Prorocentrum concavum*#	16.44 ± 9.69^{ab}	21.14 ± 7.81^{ab}	32.64 ± 20.40^{ab}	24.10 ± 14.51^{ab}	25.3 ± 7.25^{ab}	40.61 ± 28.98^{a}	
P. emarginatum*#	1.43 ± 3.50^{a}	4.09 ± 4.77^{a}	10.76 ± 9.27^{a}	$8.40\pm5.30^{\rm a}$	7.38 ± 7.61^{a}	20.14 ± 21.04^{a}	
P. fukuyoi#	18.45 ± 11.80^{a}	15.75 ± 13.79^{a}	6.17 ± 5.45^{a}	$8.97\pm6.60^{\rm a}$	$5.63\pm8.51^{\rm a}$	$28.27\pm15.50^{\rm a}$	
P. lima*	11.26 ± 7.59^{a}	22.77 ± 18.47^{a}	12.30 ± 6.90^{a}	23.17 ± 19.51^{a}	33.87 ± 14.49^{a}	41.79 ± 24.57^{a}	
P. rhathymum*	7.55 ± 13.05^{a}	11.014 ± 14.00^{a}	6.99 ± 7.49^{a}	$5.70\pm9.63^{\rm a}$	18.04 ± 17.32^{a}	30.02 ± 29.23^{a}	
Total dinoflagellates	$173.15 \pm 72.25^{\circ}$	203.03 ± 47.79^{bc}	$266.67 \pm 67.98^{\rm bc}$	317.02 ± 54.04^{bc}	424.63 ± 103.85^{bc}	751.82 ± 223.12^{a}	

 Table 2. List of epiphytic dinoflagellates and abundance (cells g⁻¹ wet wt of algae) for each month during July 2012-June 2013 from Jeju Island, Korea.

An '*' denotes potentially toxic species. A '#' denotes new record for Korean waters. Values are reported as averages \pm standard deviation. The letters next to each dinoflagellate abundance value indicate that values are significantly different from the others within the same row, where 'a' is the highest value and 'c' is the lowest value.

Sedgwick-Rafter counting chamber under microscope (Axioplan 2, Carl Zeiss, Oberkochen, Germany) at 20X and 40X magnifications. Cell density of epiphytic dinoflagellates expressed as cells g^{-1} wet weight of macroalgae (cells g^{-1} hereafter), following the methodology described by Delgado *et al.* [12] and Okolodkov *et al.* [40].

2. Identification of Dinoflagellates and Macroalgae

Freshly-collected living dinoflagellates were isolated by the micropipette-washing method, placed on slide glass covered with a cover slip, and the morphometric features were observed under transmitted light with bright field and phasecontrast at 400X magnification and photographed using a microscope (Axioplan 2, Carl Zeiss) equipped with a digital camera (Axiocam ERc5s, Carl Zeiss). Both the dorsal and ventral sides of each dinoflagellate were examined. Cell size and some morphometric measurements were obtained from micrographs using Carl Zeiss ZEN Lite software. Thecal plate patterns of armored dinoflagellates were identified using Calcofluor White M2R [17]. The Calcofluor stained cells were examined using an epifluorescence (violet excitation at 430 nm, blue emission at 490 nm) microscope (Axioplan 2, Carl Zeiss) equipped with a digital camera (Axiocam ICm1, Carl Zeiss). Unarmored dinoflagellates were identified based on morphological features such as body contour and proportion, cingulum displacement, sulcus extension and direction on the epitheca, and presence and location of specific organelles. Dinoflagellates were identified using previously published schemes [3, 10, 14-16, 18, 21, 36, 38, 47]. Macroalgae were identified using appropriate keys [30, 31] and cataloged.

3. Physico-Chemical Parameters Analysis

Hydrological variables such as water temperature (°C) and salinity (psu) were estimated every month during sample collection in the water column of intertidal zone of all sampling stations with a temperature-salinity meter (YSI 35, Yellow Spring Instrument, Ohio, USA).

4. Statistical Analysis

Epiphytic dinoflagellate abundance data (each individual species and total number of dinoflagellate species) were tested for site and macroalgal host preference using one-way ANOVA with Tukey's pair wise comparisons. Dinoflagellate abundance data were also tested for significant correlation (Pearson) with environmental variables (temperature, salinity). All statistical tests were conducted using Microsoft Excel and Graph Pad InStat ver.3 at an alpha level of 0.05.

III. RESULTS AND DISCUSSION

1. Epiphytic Dinoflagellate Species Composition

From the six study sites, ten species in five genera of epiphytic dinoflagellates were identified; of these, eight are potentially toxic species and four are first reported in Korean waters (Table 2). The number of species encountered in this study is comparable to values reported in other studies conducted around the world. For example, Faust [13] reported 16 species in sandy environments around Belize. Parsons and Preskitt [41] reported 26 benthic dinoflagellate species from Hawaiian coastal waters. Four of the species found in this study, have been reported previously in Jeju Island: Coolia malayensis [27], Ostreopsis ovata [28], Prorocentrum rhathymum [33], and P. lima [45]. Amphidinium carterae, A. operculatum, P. concavum, P. emarginatum, and P. fukuyoi are reported as present in Jeju coastal waters for the first time. Although Gambierdiscus caribaeus was reported from Jeju Island in previous study [26], Gambierdiscus sp. found in this study was not identified up to species level. Several of these dinoflagellates (e.g., Gambierdiscus sp., P. concavum, P. emarginatum, P. rhathymum, and O. ovata) have been reported from many other tropical and subtropical locations including Pacific Ocean [41], the Caribbean Sea [15], and in the Mediterranean Sea [2]. P. lima is a cosmopolitan species, found from boreal to tropical waters around the world [15, 41] and P. rhathymum commonly found in tropical and subtropical waters in the Atlantic and Pacific Ocean [15].

Eight of the encountered species in this study can be considered potentially toxic species [46], because they have been reported as toxic elsewhere [39, 52]. All species of *Prorocentrum* (except *P. fukuyoi*) in this study are reported as toxic (Table 2), known to produce okadaic acid and its derivatives, responsible of diarrheic shellfish poisoning and may be involved in ciguatera fish poisoning [37].

2. Seasonal Abundance of Epiphytic Dinoflagellates

Epiphytic dinoflagellates were found during all the months of the annual sampling cycle (July 2012-June 2013) in the intertidal zone along the coasts of Jeju Island. The average abundance of total epiphytic dinoflagellates was significantly (p < 0.05) highest (751.82 ± 223.12 cells g⁻¹) and lowest $(173.15 \pm 72.25 \text{ cells g}^{-1})$ in June (summer) and January (winter), respectively (Table 2). The abundance pattern changed in August (late summer with rainfall), when we observed abrupt decline in the number of total cells during this month. A second peak of total average abundance (650.45 \pm 225.02 cells g^{-1}) occurred in October (autumn) (Table 2). Similar seasonal pattern was reported by Delgado et al. [12] from northwestern coast of Cuba, where highest average abundance was in June 1999-2000 (1012 cells g⁻¹) and in 2000-2001 (1089 cells g^{-1}) and lowest mean concentration (21 cells g⁻¹) was in February. In 1999-2000, these authors observed abrupt decline in cells during August and September, and second peak of abundance (609 cells g^{-1}) in October.

In case of monthly variation of epiphytic dinoflagellates abundance in Jeju Island, O. ovata among the ten species was the most abundant species $(338.21 \pm 11 \text{ cells g}^{-1})$, which was reported in October (Table 2). The abundances of epiphytic dinoflagellate Ostreopsis sp. were quite lower than values reported in the previous studies: for example, Kim et al. [29]; about 9×10^3 cells g⁻¹, Parson and Preskitt [41]; 18×10^3 cells g⁻¹, Mangialajo *et al.* [35]; 2451×10^3 cells g⁻¹, Holmes *et al.* [24]; 3.3×10^3 cells g⁻¹, Aligizaki and Nikolaidis [1]; about 4.0×10^5 cells g⁻¹ for Ostreopsis ovata + O. siamensis. Gambierdiscus sp. was the least abundant species, which was found only in September (average 6.92 ± 16.97 cells g⁻¹) and October (average 6.54 \pm 6.54 cell g⁻¹) (Table 2). The abundance of Gambierdiscus sp. in the present study was extremely lower than these of Kim *et al.* [29] (about 5×10^3 cell g⁻¹); *G. toxicus* from the Gambier Islands, Pacific Ocean by Yasumoto et al. [51] $(500 \times 10^3 \text{ cells g}^{-1})$; Gambierdiscus sp. from Hawaii (average 127 cells g⁻¹) [41]; Tahiti, French Polynesia ($10.88 \times$ 10^3 cells g⁻¹) [10]. Similar to our study, low abundance of Gambierdiscus, G. toxicus was reported from Mauritius (0~4 cells g^{-1}) [25].

The average abundance of A. carterae and A. operculatum reached highest of 76.39 ± 37.21 cell g⁻¹ and 103.04 ± 37.60 cells g⁻¹, respectively in June. The abundance of these species are also lower than Kim et al. [29], wherein the authors reported a maximum abundance of 0.41×10^3 cells g⁻¹ for Amphidinium spp. from Jeju Island. Okolodkov et al. [40] reported abundance of 41172 cells g⁻¹ from Gulf of Mexico, which is higher than our study. Coolia malayensis showed highest average abundance of 109.95 ± 43.82 cells g⁻¹ in this study, whereas Kim et al. [29] reported higher abundance of 0.71×10^3 cells g⁻¹ for *Coolia* spp. Calson and Tindoll [8] recorded 1200×10^3 cells g⁻¹ for *Coolia monotis* from Virgin Island, Caribbean Sea. The average abundance of Prorocentrum concavum was also lower in our study (41.61 \pm 28.98 cells g⁻¹) compared to the study by Delgado *et al.* [12] ($<10^3$ cell g⁻¹). Parson and Preskitt [41] estimated cell densities of 0-69 cells g⁻¹ for Prorocentrum emarginatum and 3-224 cells g⁻¹ for *Prorocentrum lima*, which are more similar to our findings (*P. emarginatum*: 20.14 ± 43.82 cells g⁻¹ and *P. lima*: 41.79 ± 24.57 cells g⁻¹) but less than those reported by Delgado *et al.* [12] $(10^4 \sim 10^5 \text{ cell g}^{-1})$ and Okolodkov *et al.* [40] (29756 cells g⁻¹) of *P. lima*. Parson and Preskitt [41] recorded P. lima + P. concavum densities averaged from 0 to 598 cells g⁻¹. In this study *Prorocentrum fukuyoi* and *Prorocen*trum rhathymum average cell abundance were 28.27 ± 5.50 cells g⁻¹, and 30.02 ± 29.23 cells g⁻¹ (Table 2). Kim *et al.* [29] reported higher abundance of *Prorocentrum* spp. (0.30×10^3) cells g⁻¹) from Jeju Island than our study.

3. Spatial Abundance and Distribution of Epiphytic Dinoflagellates

Epiphytic dinoflagellates were found at all the stations during the sampling period. There was significant (p < 0.05) difference in the total cell numbers between sampling stations

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Dinoflagellates	Sehwa (46)	Hamduk (51)	Hyupjae (47)	Hamo (45)	Hwasun (43)	Sinyang (45)		
Amphidinium carterae	19.18 ± 16.49^{a}	$27.83\pm38.22^{\rm a}$	30.99 ± 16.98^{a}	15.06 ± 17.46^{a}	25.50 ± 26.05^a	31.29 ± 17.791^{a}		
A. operculatum	35.19 ± 28.71^{a}	40.68 ± 40.89^{a}	32.77 ± 16.55^{a}	36.29 ± 25.76^{a}	35.97 ± 33.45^{a}	44.95 ± 26.78^a		
Coolia malayensis	63.16 ± 42.32^{a}	97.50 ± 73.00^{a}	68.84 ± 34.85^a	68.53 ± 41.28^{a}	47.46 ± 26.41^{a}	66.25 ± 30.35^{a}		
Gambierdiscus sp.	0 ± 0	7.351 ± 16.36	0 ± 0	0 ± 0	0 ± 0	0 ± 0		
Ostreopsis ovata	$215.09 \pm 129.08^{\rm a}$	241.44 ± 150.21^{a}	191.99 ± 125.19^{b}	160.21 ± 92.81^{b}	$81.00\pm58.40^{\rm c}$	167.50 ± 99.52^{b}		
P. concavum	31.34 ± 12.97^{a}	42.54 ± 24.03^{a}	28.27 ± 13.94^{a}	24.24 ± 14.19^{a}	12.60 ± 12.60^{a}	$13.68\pm12.37^{\mathrm{a}}$		
P. emarginatum	$9.62\pm7.74^{\rm a}$	$16.55\pm18.08^{\mathrm{a}}$	$5.21\pm7.57^{\rm a}$	$6.48 \pm 8.41^{\mathrm{a}}$	5.96 ± 13.17^{a}	3.98 ± 6.58^a		
P. fukuyoi	18.51 ± 12.52^{a}	21.94 ± 15.71^{a}	16.49 ± 11.57^{a}	$7.34\pm10.14^{\rm a}$	$0.20\pm0.72^{\rm b}$	19.01 ± 12.18^{a}		
P. lima	35.64 ± 32.66^{a}	39.91 ± 25.32^{a}	16.79 ± 15.06^{ab}	10.80 ± 12.99^{ab}	$6.44\pm8.69^{\rm b}$	11.53 ± 17.50^{ab}		
P. rhathymum	$2.34\pm5.06^{\rm b}$	7.22 ± 10.19^{ab}	25.19 ± 18.99^{a}	14.07 ± 13.39^{a}	25.44 ± 19.69^{a}	$0.94 \pm 3.26^{\circ}$		
Total Dinoflagellates	426.40 ± 222.01^{a}	547.91 ± 315.37^{a}	423.82 ± 176.98^{a}	348.01 ± 157.25^{a}	232.59 ± 144.93^{b}	358.10 ± 171.54^{a}		

Table 3. Abundance (cells g⁻¹ wet wt of algae) of epiphytic dinoflagellate species at each of the six sampling sites from Jeju Island during July 2012-June 2013.

Values are reported as averages \pm standard deviation. The numbers in parentheses next to each site represent the number of samples analyzed. The letters next to each dinoflagellate abundance value indicate that values are significantly different from the others within the same row, where 'a' is the highest value and 'c' is the lowest value.

(Table 3). Highest total abundance occurred at Hamduk (St 2) with mean value of 547.91 ± 315.37 cells g⁻¹. Lowest total abundance of epiphytic dinoflagellates was found at Hwasun (St 5), with mean concentrations of 232.59 ± 1441.93 cells g⁻¹ (Table 2). At Hamduk (St 2), all the epiphytic dinoflagellates showed highest mean abundance, except Amphidinium carterae and A. operculatum occurred at maximum mean cell abundance at Sinyang (St 6) (Table 3). Among all the sampling stations, comparatively lower abundance of epiphytic dinoflagellates at Hamo (St 4) and Hwasun (St 5) stations (more exposed coasts located at south-western part of Jeju Island) might be related with species interactions of dinoflagellates with physical and biological environment and also macroalgal abundance. This observation agrees well with Kim et al. [29], who also found lower abundance of epiphytic dinoflagellates near those areas. Shaking effect due to turbulent water at the open coast (physical-biological interaction) and/or diverse bi-species interaction among different macroalgae-epiphyte combinations may limit or support their success [7].

4. Dynamics of Water Temperature and Salinity, and Their Relation to Abundance of Dinoflagellates

The water temperature exhibited a predictable seasonal fluctuation with the range of 10.9° C to 26.3° C. Monthly average water temperature for all stations was highest ($26.016 \pm 0.33^{\circ}$ C) in July 2012 (summer) and lowest ($12.98 \pm 1.44^{\circ}$ C) in January 2013 (winter) (Fig. 2A). During the study period, salinity of the coastal seawaters in intertidal zone of Jeju Island ranged from 23.6 to 34.8 psu. For all the sampling stations, monthly average lowest salinity (26.33 ± 1.94 psu) was recorded in August 2012 (summer) and the highest salinity (33.91 ± 0.83 psu) was in April 2013 (spring) (Fig. 2B).

Total dinoflagellate abundance, C. malayensis, O. ovata, Gambierdiscus sp., P. concavum, P. emarginatum, P. fukuyoi and P. rhathymum abundance were significantly correlated

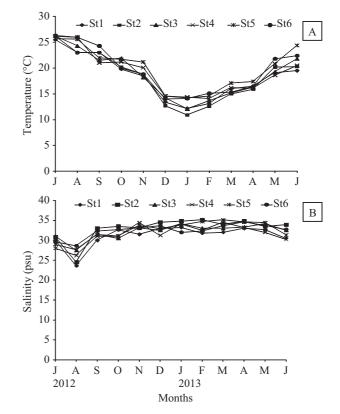


Fig. 2. Monthly variations in water temperature (A) and salinity (B) in the intertidal zone from July 2012 to June 2013 at different sampling stations of Jeju Island, Korea.

with water temperature and salinity (Table 4). A. carterae was the only dinoflagellate to be not significantly correlated with temperature and salinity. A. operculatum did not show significant correlation with the salinity and P. lima was not significantly correlated with temperature variation in this study. Lower abundance of epiphytic dinoflagellates during winter

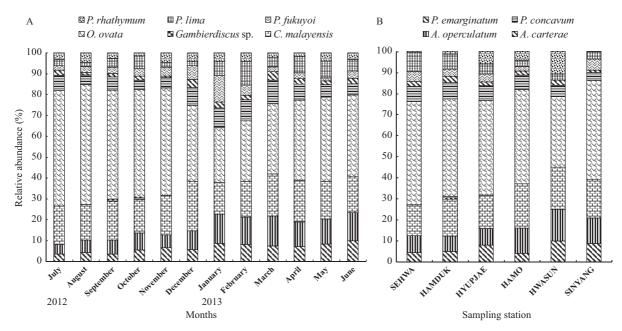


Fig. 3. A. Relative abundance of epiphytic dinoflagellate species by month from July 2012 to June 2013. B. Relative abundance of epiphytic dinoflagellates at each station from July 2012 to June 2013.

Table 4. Pearson correlation analysis results between epi-
phytic dinoflagellates abundance and water tem-
perature and salinity parameters at all stations
during July 2012-June 2013 from Jeju Island,
Korea.

Dinoflagellates	Temperature (°C)	Salinity (psu)		
Amphidinium carterae	ns	ns		
A. operculatum	0.18 (0.01)*	ns		
Coolia malayensis	0.43 (0.00)**	0.00 (0.00)**		
Gambierdiscus sp.	0.29 (0.00)**	-0.09 (0.00)**		
Ostereopsis ovata	0.75 (0.00)**	-0.33 (0.00)**		
P. concavum	0.34 (0.03)*	0.12 (0.02)*		
P. emarginatum	0.23 (0.00)**	0.08 (0.00)**		
P. fukuyoi	-0.12 (0.05)*	0.17 (0.00)**		
P. lima	ns	0.17 (0.00)**		
P. rhathymum	0.48 (0.01)*	-0.23 (0.00)**		
Total average	0.59 (0.00)**	-0.14 (0.00)**		

Non-significant results are denoted by 'ns' for each dinoflagellate species. The correlation coefficient and corresponding *p*-value (in parentheses) at $p < 0.05^*$ and $p < 0.001^{**}$ is given for significant results.

months might be due to lower temperature recorded in Jeju Island. Higher water temperatures could be a promoting factor for the growth of macroalgae and epiphytic dinoflagellates [12]. Lower abundance seems to be related to decreasing salinity due to rainfall in August, which agrees well with the observation in the northwestern coast of Cuba [12]. In September and October, the increase of epiphytic dinoflagellates in Jeju Island probably depended on several environmental factors acting at the same time [8].

Our results were found to be comparable to those observed in the Virgin Islands, where all seven toxic epiphytic dinoflagellate species were positively or negatively correlated with water temperature [8]. In east Tasmanian waters, a decrease in temperature was associated with a decline in dinoflagellate numbers through winter [42]. In contrast, in southwestern Puerto Rico, two dominant epiphytic dinoflagellate species were not strongly correlated with temperature [5]. In Queensland, Australia, periodicity in the G. toxicus abundance did not seem to be related directly to temperature, and complex substrate interactions and other unknown factors appear to be involved [20]. In the NW Mediterranean, no significant correlations were observed between epiphytic dinoflagellates and water temperature; however, the epiphytic dinoflagellate assemblage demonstrated a clear seasonality [50]. High harmful dinoflagellates concentrations in May and October were related to physicochemical conditions in northwestern coast of Cuba [12]. The mechanisms that trigger species abundance are unclear, but changes in the hydrodynamic regime may be involved [50].

5. Relative Abundance of Epiphytic Dinoflagellates

In this study, *Ostreopsis ovata* predominated in abundance and spatial-temporal distribution of epiphytic dinoflagellates and was perennially present. Highest (57.4%) and lowest (26.1%) relative abundance of *O. ovata* occurred in August 2012 and January 2013, respectively, when low densities on the whole epiphytic dinoflagellates occurred in all stations. The relative abundances of other species were <10%, except for *Coolia malayensis* and *Amphidinium operculatum* that reached a highest relative abundance (23.48% and 14.33%) in December and March 2013, respectively (Fig. 3A). Considering the relative abundance of each dinoflagellate species in different stations, *O. ovata* was also the dominant species (33.6% to 49.1%) at all sampling stations followed by *C. malayensis* (14.5% to 21%). The remaining species were <10%, except for *A. operculatum*, which reached a maximum relative abundance of 15% at Hwasun (St 5) (Fig. 3B).

An association, consisting of Ostreopsis siamensis, O. lenticularis, O. ovata, Prorocentrum lima, P. compressum and Coolia monotis, has been recorded in northern New Zealand [9]. The dominant species, O. siamensis, accounted for 64% to 85% of the total epiphytic flora during summer [9] which is more or less similar to our findings for this study. Bomber et al. [6] reported P. lima species during the entire year in the Florida Keys, being more abundant from November to May. Heil et al. [23] reported P. lima species as the most abundant along Australian shores and associated with ciguatera. Delgado et al. [12] found P. lima as the dominant species (<50%) at all sampling stations in northwestern coast of Cuba followed by G. toxicus (8 to 33%) and remaining species were lower than 8%. In this study, P. lima was never found as dominant species. Hurbungs et al. [25] reported Amphidinium sp. dominated (32.5%) over other species followed by *Prorocentrum* sp. (27.3%) and C. monotis (21.4%) in Mauritius.

6. Host Preference of Epiphytic Dinoflagellates

We found total 30 species of macroalgae belonging to 21 genera under Rhodophyta, Phaeophyta and Chlorophyta (Table 5). Various species of macroalgae (Rhodophyta, Phaeophyta, Chlorophyta, and Cyanophyta) have been reported as host for different numbers of epiphytic dinoflagellates [49].

Among the ten epiphytic dinoflagellates, *Amphidinium carterae* and *Ostreopsis ovata* were found on all the macroalgal samples. Abundance of *A. carterae* was highest (16.94 ± 17.71 cells g⁻¹) on Pheophyte (*Dictyopteris prolifera*). Kim *et al.* [29] observed *Amphidinium* spp. with seven macroalgal species (*Cladophora wrightiana, Sargassum* sp., *Dictyopteris divaricata, Chordaria flagelliformis, Padina arborescens,* and *Martensia* sp.) with maximum cell density on *Martensia* sp. (406 cells g⁻¹). From these seven macroalgal species, *Chordaria flagelliformis, Padina arborescens* were not found during this study.

O. ovata cell abundance was overall higher for most of the macroalgal host with the highest abundance $(102.07 \pm 17.71 \text{ cells g}^{-1})$ on Chlorophyte *Cladophora wrightiana*. *Amphidinium operculatum* cells were recorded from all the macroalgal samples with highest abundance $(22.66 \pm 17.71 \text{ cells g}^{-1})$ on Rhodophyte *Chondrus ocelatus* but it was only absent on *Jania adhaerens*. *O. ovata* were not found attached on *Codium fragile* by Kim *et al.* [29], which disagree with present study. The highest density $(5.9 \times 10^5 \text{ cells g}^{-1})$ detected for *Ostreopsis* sp. on *Halopteris scoparia* by the study of Vila *et al.* [50].

Coolia malayensis also preferred all macroalgae except one Chlorophyte *Codium fragile* which agreed with the observation by Kim *et al.* [29]. In this study, *C. malayensis* found on all macroalgae reported by Kim *et al.* [29], except *C. flagelliformis* and *P. arborescens. C. malyensis* highest cell density on *Hizikia fusiformis* (42.21 ± 33.15 cell g⁻¹) from this study differed with Kim *et al.* [29], who found highest cell density (710 cells g⁻¹) of *Coolia* spp. on Rhodophyte *Martensia* sp. Carlson and Tindol [8] found *C. monotis* density of 1.2×10^6 cells g⁻¹ in Virgin Islands, which is very high compared to Jeju Island, Korea.

In our study, *Gambierdiscus* sp. was exceptionally absent on all macroalgae and only exclusively found attached on Rhodophyte *Gelidium amansii* with low abundance, whereas *Gambierdiscus* spp. were attached all the macroalgae, except *Codium fragile* and *Sargassum siliquastrum*, collected by Kim *et al.* [29]. *G toxicus* was estimated to be 5.0×10^5 cells g⁻¹ on Rhodophyte *Jania* in a Gambier Island reef [51]. Depending on the geographic region, *G toxicus* has been shown to prefer different macroalgal host species and found with more than 50 algal genera [8, 11].

Generally, the species under the genus Prorocentrum were less abundant on Chlorophyte macroalgal species in this study. *P. concavum* was present on all the Rhodophytes, while it was absent on one Pheaephyte (Ecklonia cava) and two Chlorophytes (Codium fragile and Ulva pertusa). Cell abundance of this epiphytic dinoflagellate was always below 15 cells g⁻¹. P. emarginatum never preferred any of the Chlorophytes and also absent on Rhodophyte, Wrangelia tanegana and Pheophytes, Sargassum macrocarpum and S. siliquastrum. P. fu*kuyoi* abundance was highest $(13.17 \pm 11.35 \text{ cells g}^{-1})$ on Rhodophyte Chondrus ocelatus but cell abundance was lower for most of the macroalgal host. P. lima and P. rhathymum were found with maximum cell concentration (12.49 \pm 8.42 and 22.04 \pm 21.96 cells g⁻¹) on Pheophyte Sargassum horneri and S. confusum, respectively. Kim et al. [29] observed Prorocentrum spp. with eight macroalgal species (U. pertusa, E. caba, Sargassum sp., D. divaricata, C. flagelliformis, P. arborescens, Martensia sp., Gelidium amansii, Corallina sp.) with maximum density (304 cells g^{-1}) on Martensia sp. At Virgin Islands, *P. mexicanum* cell density was found at $1.5 \times$ 10^6 cells g⁻¹ [8].

Generally, no macroalgal species appeared to be an overall best or worst host for specific epiphytic dinoflagellates. Additionally, preferences were not consistent among the dinoflagellates. Significant differences in epiphytic densities between macroalgae were not observed in this study, which agrees with Lobel *et al.* [34] and Bomber *et al.* [7].

The availability of macroalgal substrates and water movement may affect the spatial distribution of epiphytic dinoflagellates. Beside these, environmental variables and nutrients from macroalgae could be important temporary factors [49]. Therefore, the temporal patterns should be interpreted with respect to the succession of host macroalgae [32]. In addition, macroalgal surface area and ash content appear to be regulating factors of epiphytic dinoflagellate population density [6].

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Table 5. Average abundance (cells g⁻¹ wet wt of algae) of epiphytic dinoflagellates on each of the macroalgal species during July 2012-June 2013 from Jeju Island.

Algal host species	Total	AC	AO	СМ	GM	00	PC	PE	PF	PL	PR
Rhodophyta											
Chondrus canaliculatus (12)	121.03 ± 171.37	8.20 ± 9.41	14.57 ± 14.81	7.83 ± 10.69	0 ± 0	48.77 ± 42.17	5.19 ± 5.14	2.13 ± 4.81	4.11 ± 6.86	9.35 ± 13.04	1.1 ± 2.69
Chondrus ocelatus (5)	64.12 ± 60.37	4.78 ± 7.81	22.66 ± 30.82	26.81 ± 53.99	0 ± 0	35.16 ± 53.35	12.85 ± 12.57	1.67 ± 3.74	13.17 ± 11.35	11.32 ± 15.05	0 ± 0
Coralina pilulifera (7)	53.53 ± 86.44	2.82 ± 5.30	13.16 ± 10.93	7.24 ± 12.5	0 ± 0	40.33 ± 34.06	2.76 ± 3.99	0.74 ± 1.96	0 ± 0	0 ± 0	9.41 ± 9.82
Galaxaura apiculata (9)	94.46 ± 140.48	3.69 ± 1.51	4.60 ± 2.97	25.79 ± 18.33	0 ± 0	48.48 ± 49.57	11.47 ± 1692	10.54 ± 11.42	0 ± 0	0.35 ± 0.5	0 ± 0
Galaxaura falcate (4)	46.05 ± 60.93	6.73 ± 3.89	14.65 ± 12.99	23.11 ± 25.09	0 ± 0	49.98 ± 69.71	6.16 ± 8.08	7.12 ± 7.25	0 ± 0	5.37 ± 6.78	1.99 ± 3.99
Gelidium amansii (24)	296.44 ± 432.66	8.05 ± 8.24	6.29 ± 8.78	21.24 ± 14.51	3.5 ± 11.6	61.16 ± 41.58	4.71 ± 4.85	3.39 ± 5.44	2.03 ± 4.89	9.95 ± 9.42	3.70 ± 11.82
Gracilaria sp. (9)	88.99 ± 99.73	10.45 ± 9.94	10.50 ± 13.06	15.68 ± 10.89	0 ± 0	37.78 ± 11.51	9.82 ± 3.52	2.76 ± 2.3	3.15 ± 4.68	0 ± 0	0 ± 0
Gracilaria vermiculophylla (2)	17.27 ± 31.31	3.82 ± 5.4	4.16 ± 5.88	14.13 ± 1.66	0 ± 0	51.77 ± 40.26	3.24 ± 4.58	3.24 ± 4.58	2.05 ± 2.89	0 ± 0	0 ± 0
Grateloupia asiatica (6)	60.29 ± 85.27	3.06 ± 3.80	12.82 ± 8.21	21.59 ± 13.59	0 ± 0	45.76 ± 21.93	7.82 ± 6.01	1.19 ± 3.21	0.81 ± 0.2	4.81 ± 0.89	2.59 ± 5.23
Hypnea charoides (7)	75.72 ± 108.85	4.26 ± 5.33	7.90 ± 5.22	28.07 ± 17.38	0 ± 0	48.28 ± 51.37	10.1 ± 5.82	0.81 ± 2.16	2.77 ± 1.58	5.95 ± 2.26	0 ± 0
Jania adhaerens (12)	157.55 ± 283.07	0.73 ± 1.97	0 ± 0	27.23 ± 33.06	0 ± 0	75.44 ± 48.27	13.74 ± 12.26	0.26 ± 0.90	4.42 ± 10.59	9.46 ± 11.19	0 ± 0
Martensia sp. (6)	87.49 ± 127.84	4.11 ± 6.50	21.9 ± 19.15	27.42 ± 11.55	0 ± 0	69.64 ± 68.63	3.41 ± 4.93	5.33 ± 5.17	4.89 ± 7.18	6.56 ± 8.55	2.53 ± 4.48
Plocamium telfairiae (25)	350.33 ± 22.35	4.95 ± 9.22	8.37 ± 9.45	23.71 ± 19.24	0 ± 0	74.58 ± 65.29	12.17 ± 8.90	2.33 ± 5.08	5.01 ± 2.64	11.22 ± 17.85	0 ± 0
Pterocladiella capillacea (4)	51.85 ± 59.40	5.09 ± 5.88	12.99 ± 5.95	22.72 ± 29.78	0 ± 0	50.45 ± 40.0	11.08 ± 2.17	7.49 ± 14.98	9.65 ± 7.26	10.15 ± 11.54	0 ± 0
Wrangelia tanegana (11)	86.16 ± 109.29	9.38 ± 5.68	10.23 ± 4.14	9.95 ± 6.25	0 ± 0	33.46 ± 5.49	5.87 ± 5.26	0 ± 0	2.31 ± 2.11	7.10 ± 5.96	0 ± 0
Phaeophyta											
Champia expansa (3)	38.32 ± 47.12	16.66 ± 21.41	18.51 ± 20.0	30.13 ± 32.49	0 ± 0	47.61 ± 52.60	2.46 ± 3.74	3.87 ± 5.05	4.42 ± 6.70	4.05 ± 6.14	0 ± 0
Colpomenia sinuosa (3)	21.37 ± 28.75	2.48 ± 2.36	2.51 ± 2.36	11.1 ± 7.98	0 ± 0	32.20 ± 26.06	4.89 ± 4.38	8.27 ± 10.59	0 ± 0	8.14 ± 14.11	1.63 ± 2.82
Dictyopteris prolifera (4)	52.19 ± 62.96	16.94 ± 17.71	15.89 ± 10.64	37.66 ± 18.43	0 ± 0	43.17 ± 42.97	6.95 ± 12.47	7.75 ± 13.08	0 ± 0	0 ± 0	0.94 ± 1.89
Dictyopteris divaricate (3)	15.76 ± 19.36	3.58 ± 0.58	15.81 ± 10.20	14.84 ± 9.71	0 ± 0	12.36 ± 12.54	1.03 ± 1.79	1.48 ± 2.56	0 ± 0	3.43 ± 5.95	0 ± 0
Ecklonia cava (22)	60.29 ± 107.33	10.45 ± 13.47	13.03 ± 11.70	1.99 ± 8.39	0 ± 0	0.38 ± 1.17	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Hizikia fusiformis (7)	98.78 ± 161.40	4.31 ± 5.99	0.73 ± 0	42.21 ± 33.15	0 ± 0	69.39 ± 38.96	9.68 ± 3.7	2.30 ± 2.60	3.2 ± 5.80	3.20 ± 5.56	6.52 ± 12.09
Sargassum confusum (5)	40.93 ± 42.37	1.02 ± 2.29	6.45 ± 5.52	18.41 ± 9.06	0 ± 0	19.47 ± 11.80	4.85 ± 3.20	4.57 ± 4.36	5.02 ± 5.66	0 ± 0	22.04 ± 21.96
Sargassum horneri (7)	91.39 ± 119.57	6.84 ± 6.41	14.74 ± 10.93	24.36 ± 14.5	0 ± 0	56.74 ± 58.26	7.92 ± 6.19	0.93 ± 2.47	5.65 ± 6.52	12.49 ± 8.42	0.85 ± 2.24
Sargassum macrocarpum (10)	112.40 ± 159.05	1.58 ± 3.48	9.26 ± 14.07	30.40 ± 34.52	0 ± 0	48.80 ± 36.88	10.51 ± 10.01	0 ± 0	4.66 ± 7.37	3.69 ± 4.88	4.52 ± 10.11
Sargassum siliquastrum (9)	78.66 ± 102.28	1.77 ± 3.55	4.66 ± 4.93	16.83 ± 8.71	0 ± 0	37.10 ± 24.41	11.57 ± 6.75	0 ± 0	3.94 ± 3.05	9.43 ± 3.25	2.52 ± 3.00
Sargassum thunbergii (22)	195.79 ± 215.46	7.61 ± 6.69	11.59 ± 8.72	23.03 ± 18.34	0 ± 0	25.37 ± 46.79	0.80 ± 2.72	0.29 ± 1.33	2.34 ± 4.5	0.69 ± 2.23	18.41 ± 16.66
Chlorophyta											
Cladophora wrightiana (4)	54.04 ± 125.76	10.09 ± 6.89	11.85 ± 5.92	5.50 ± 111.01	0 ± 0	102.07 ± 44.15	5.59 ± 11.18	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Codium fragile (4)	21.57 ± 30.60	12.38 ± 8.51	12.79 ± 6.57	0 ± 0	0 ± 0	20.98 ± 34.82	0 ± 0	0 ± 0	0 ± 0	0 ± 0	7.76 ± 15.53
Enteromorpha linza (5)	50.29 ± 85.86	4.90 ± 2.63	8.71 ± 4.18	4.07 ± 9.10	0 ± 0	57.48 ± 16.31	4.05 ± 9.05	0 ± 0	10.73 ± 3.25	10.63 ± 11.91	0 ± 0
Ulva pertusa (26)	223.58 ± 415.59	10.0 ± 4.69	12.95 ± 8.63	3.25 ± 10.75	0 ± 0	52.07 ± 45.57	0 ± 0	0 ± 0	7.22 ± 8.52	0.44 ± 2.25	0.30 ± 1.57

AC = A. carterae, AO = A. operculatum, CM = C. malayensis, GM = Gambierdiscus sp., OO = O. ovata, PC = P. concavum, PE = P. emarginatum, PF = P. fukuyoi, PL = P. lima and PR = P. rhathymum. Values are reported as averages \pm standard deviation. The numbers in parentheses next to each algal host species name represent the number of samples analyzed.

IV. CONCLUSION

The results of this study indicate that, (1) several potentially toxigenic dinoflagellate species are present in Jeju Island; (2) The epiphytic dinoflagellate assemblage demonstrated that they were being more abundant in September and October 2012 (autumn), and June 2013 (summer) than the other months; (3) The abundance of epiphytic dinoflagellates was appeared to be higher at Hamduk (St 2) and lower at Hamo (St 4) and Hwasun (St 5) in Jeju Island; (4) The population density of most of the epiphytic dinoflagellates was found to be significantly correlated with the variation of both water temperature and salinity, except *A. carterae*, *A. operculatum* and *P. lima*; (5) Epiphytic dinoflagellates did not show any significant preference for macrophyte species.

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