Spatial and seasonal distributions of the phototrophic dinoflagellate *Biecheleriopsis adriatica* (Suessiaceae) in Korea: quantification using qPCR

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The phototrophic dinoflagellate *Biecheleriopsis adriatica* is a small suessioid species characterized by a fragile thin wall. Although the morphology of this dinoflagellate is well established, there is currently little information available on its distribution and the environmental factors that influence this distribution. Thus, to investigate the spatial and seasonal distributions of the vegetative cells of *B. adriatica* in Korean waters, surface water samples were collected on a seasonal basis from 28 stations in the East, West, and South Sea of Korea and Jeju Island from April 2015 to October 2018, and abundances of the vegetative cells of *B. adriatica* were quantified using quantitative real-time polymerase chain reactions, for which we developed the species-specific primer and probe set. Simultaneously, major environmental parameters, including temperature, salinity, nutrient concentrations, and dissolved oxygen concentrations were measured. The vegetative cells of *B. adriatica* were detected at 20 of the 28 sampling stations: 19 stations in summer and 6 in autumn, although from no stations in either spring or winter. The ranges of water temperature and salinity at sites where this species was detected were 17.7-26.4°C and 9.9-34.3, respectively, whereas those of nitrate and phosphate concentrations were not detectable-96.2 and 0.18-2.66 µM, respectively. Thus, the sites at which this species is found are characterized by a narrow range of temperature, but wide ranges of salinity and concentrations of nitrate and phosphate. The highest abundance of the vegetative cells of *B. adriatica* was 41.7 cells mL⁻¹, which was recorded in Jinhae Bay in July 2018. In Jinhae Bay, the abundance of vegetative cells was significantly positively correlated with the concentration of nitrate, but was negatively correlated with salinity. On the basis of these findings, it appears that the abundance of *B. adriatica* vegetative cells shows strong seasonality, and in Jinhae Bay, could be affected by the concentrations of nitrate.

Key Words: abundance; molecular method; nationwide distribution; protist; Suessiaceae

INTRODUCTION

Phototrophic dinoflagellates are a major component of marine ecosystem (Hallegraeff 1993, Taylor et al. 2008,

Selina and Levchenko 2011, Jeong et al. 2013, 2017). These algae play diverse roles in marine ecosystems as

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primary producers, prey for a wide range of predators, predators on numerous different prey items, parasites, and symbiotic partners (Park et al. 2004, Tillmann 2004, Jeong et al. 2010, Hansen 2011, Johnson 2015, Stoecker et al. 2017, Kang et al. 2018, LaJeunesse et al. 2018). Many phototrophic dinoflagellate species form red tides or harmful algal blooms that can often cause extensive mortalities among marine organisms, and subsequently considerable losses in aquaculture industries (Shumway 1990, Landsberg 2002, Park et al. 2013, Adolf et al. 2015, Menden-Deuer and Montalbano 2015). The abundance of a dinoflagellate species at a given time and in a specific area is a critical parameter in understanding its bloom dynamics (e.g., Jeong et al. 2015). Moreover, some dinoflagellate species can become invasive, thereby disrupting the balance of marine ecosystems (Hallegraeff 1998, Olenina et al. 2010, Telesh et al. 2016). Therefore, the presence of invasive dinoflagellates in a particular country is of critical concern to scientists and those employed in the aquacultural industries of that country.

In many marine ecosystems, there are typically large spatial and temporal variations in the abundance of dinoflagellate species (Warner et al. 2006, Litaker et al. 2010, Jeong et al. 2013, 2017). The relatively rapid growth of dinoflagellates through binary division (Smayda 1997, Jeong et al. 2010, 2015), together with their high sensitivity to certain environmental parameters, such as water temperature, light intensity, and nutrient concentrations (Heaney and Eppley 1981, Singh and Singh 2015, Lee et al. 2017a, Jeong et al. 2018b), and intense competition with other microalgal groups, may cause considerable variations in their abundance (Smayda and Reynolds 2003, Kremp et al. 2008, Jeong et al. 2018a). Thus, to understand ecological roles of a dinoflagellate in marine ecosystems, its spatial and temporal distributions and environmental factors affecting the distributions should be explored.

Species of dinoflagellate in the order Suessiales are characterized by a cell surface covered with numerous thin plates (Lindberg et al. 2005, Siano et al. 2009, Jang et al. 2015). Owing to small cell sizes and fragile thin walls, there is a difficulty in quantifying the abundance, and thus the distribution, of many suessoid dinoflagellate species, and accordingly, to date there has been limited information available regarding the abundance and distribution of these species. In 2009, *Biecheleriopsis adriatica* was reported as a new genus and species in the order Suessiales (Moestrup et al. 2009). This species has a type E eyespot and a single elongated apical vesicle, which are key characteristics of the family Suessiaceae; however,

unlike other genera belonging to this family, the cells of *B. adriatica* possess a nuclear fibrous connective and a distinct tongue-like process in the left ventral corner of the asymmetric hyposome (Moestrup et al. 2009, Takahashi et al. 2014, Jang et al. 2015, Luo et al. 2015, Kang and Wang 2018). Vegetative cells of this species have been found in the Adriatic Sea and in the coastal waters of Korea, China, Japan, the Philippines, Singapore, and Palau Island (Moestrup et al. 2009, Takahashi et al. 2014, Jang et al. 2015, Luo et al. 2015, Benico et al. 2019), whereas resting cysts have been found in sediments of the China and Black seas (Luo et al. 2015, Dzhembekova et al. 2018, Kang and Wang 2018). To date, there has been only a single study reporting the abundance of the vegetative cells of *B. adriatica* (Benico et al. 2019) in blooms dominated by the phototrophic dinoflagellate *Takayama* sp. at Bolinao in the Philippines, which was quantified using a microscopic enumeration method. However, to the best of our knowledge, there have as yet been no studies that have examined the seasonal or regional distribution of *B. adriatica*. To understand its ecological roles in marine ecosystems, the spatial and / or seasonal distribution of the vegetative cells of *B. adriatica* should be explored. Given that the cells of *B. adriatica* are small (equivalent spherical diameter = approximately 10 μm) and have fragile thin plates (Jang et al. 2015), and that there are some suessoid dinoflagellates with morphologies similar to that of *B. adriatica*, even when observed using scanning electron microscope (e.g., Kang et al. 2011), quantification of the abundance of the vegetative cells of *B. adriatica* should ideally be performed using one of the molecular techniques that are often used for small dinoflagellate species (Bowers et al. 2000, Godhe et al. 2008, Correa et al. 2009, Lee et al. 2019).

In the present study, to explore spatial and seasonal distributions of the vegetative cells of *B. adriatica* in Korea, its abundance was quantified in the surface waters of 28 stations, located in the East, West, and South Sea of Korea and Jeju Island in four seasons from April 2015 to October 2018, using quantitative real-time polymerase chain reactions (qPCR) and newly designed *B. adriatica*-specific primer-probe set. Simultaneously, major environmental parameters such as temperature, salinity, nutrient concentrations, and dissolved oxygen (DO) concentrations were determined. The results of the present study provide a basis on understanding the spatial and temporal distributions of *B. adriatica* and critical environmental factors affecting the distributions.

MATERIALS AND METHODS

Preparation of experimental organisms

Cells of *B. adriatica* were isolated from plankton samples collected from the surface waters off the coast of Tongyoung, Korea, using plankton samplers in August 2006, when the water temperature and salinity were 28.0°C and 31.0, respectively (Jang et al. 2015). The collected samples were screened using a 154-µm Nitex mesh. A clonal culture of *B. adriatica* BATY06 was established using two consecutive single-cell isolations (Jang et al. 2015). When the concentration of *B. adriatica* had increased sufficiently, the volume was increased to 32, 270, and 500 mL in PC bottles containing fresh f/2 medium. The bottles were filled to capacity with fresh f/2 seawater medium, capped, and placed on a shelf at 20°C, illuminated with an irradiance of 20 µmol photons m⁻² s⁻¹ provided by cool white fluorescent lights, under a 14 : 10 h light : dark cycle. Only cultures in the exponential growth phase were used for observations.

To determine whether the vegetative cells of *B. adriatica* can be distinguished from those of other dinoflagellates having similar morphologies in fixed samples, the morphological characters of vegetative cells of *B. adriatica* BATY06 were compared with those of the vegetative cells of *Ansanella granifera* AGSW10 (Jeong et al. 2014a), *Biecheleria cincta* BCSW0906 (Kang et al. 2011), *Pelagodinium bei* RCC3655 (obtained from the Roscoff Culture Collection, France) and *Paragymnodinium shiwhaense* PSSH0605 (Kang et al. 2010), *Effrenium voratum* SvFL1 (Jeong et al. 2014b), *Gymnodinium smaydae* GSSW10 (Kang et al. 2014), and *Yihiella yeosuensis* YY1405 (Jang et al. 2017), and the American strain of *Pfiesteria piscicida* CCMP2091 (obtained from the National Center for Marine Algae and Microbiota, USA) using both living cells and those fixed with fixatives. For microscopic observation of living vegetative cells, a 10-mL aliquot was taken from a 250-mL bottle containing each dinoflagellate species and transferred to a 30-mL flask. One-hundred-microliter aliquots were then taken from the flask and transferred to a confocal dish (35-mm in external diameter with a hole diameter of 13 mm; SPL, Pocheon, Korea). Cells of each species in the confocal dish were photographed using a digital camera (Zeiss AxioCam HRc5; Carl Zeiss Ltd., Göttingen, Germany) attached to an inverted microscope at a magnification of ×400. Similarly, for microscopic observation of fixed vegetative cells, a 10-mL aliquot was taken from a 250-mL bottle containing each dinoflagellate species and transferred to a 30-mL flask. Five-milliliter

Fig. 1. Map showing the locations of sampling stations in the study area in Korea. The circles indicate the sampling stations. West Sea: AS, Ansan; DAJ, Dangjin; TA, Taean; MGP, Mageompo; SCN, Secheon; KS, Kusan; BA, Buan; MP, Mokpo. South Sea: BS, Busan; DDP, Dadaepo; JH, Jinhae; MS, Masan; TY, Tongyoung; YS, Yeosu; KY, Kwangyang; GH, Goheung; JAH, Jangheung. East Sea: SC, Sokcho; JMJ, Juminjin; DH, Donghae; UJ, Uljin; PH, Pohang; US, Ulsan. Jeju: AW, Aewol; SGP, Seogwipo; WM, Wimi; SS, Seongsan; GN, Gimnyeong.

aliquots were then taken from the flask, transferred to a 20-mL scintillation vial, and fixed with 5% Lugol's solution (final concentration). Furthermore, 1-mL aliquots were taken from flasks, transferred to a 20-mL scintillation vial, and fixed with 1% formalin (final concentration). One-hundred-microliter aliquots were taken from the vial and transferred to a confocal dish. Cells of each species in the confocal dish were photographed using the digital camera attached to the inverted microscope at a magnification of ×400.

Hydrographical properties

Using a clean bucket, surface water samples were collected from 28 stations located in the East, West, and South Sea of Korea, and Jeju Island, Korea, in April, July, and October of 2015; January, March, June-July, September-October, and December of 2016; March, July, and October of 2017; and January-February, March, July, and October of 2018 (Fig. 1).

The temperature, salinity, and DO for each sample were measured using an YSI Professional Plus instrument (YSI Inc., Yellow Springs, OH, USA). The water samples for nutrient concentration analysis were gently filtered through GF/F filters (Whatman Inc., Floreham Park, NJ, USA) and stored at -20°C until the concentrations of nitrate plus nitrite (NO₃ + NO₂, hereafter NO₃), phosphate (PO₄), and silicate (SiO₂) were measured using a nutrient auto-analyzer system (QuAatro; Seal Analytical GmbH, Norderstedt, Germany).

Design of TaqMan probe and primer set

Using the internal transcribed spacer (ITS) rDNA sequences of strain of *B. adriatica* and related dinoflagellates available from GenBank, the ITS sequence of *B. adriatica* BATY06 obtained from polymerase chain reaction amplification was aligned using the program MEGA v.4. To determine the unique sequences and to develop a *B. adriatica*-specific qPCR assay, manual searches of the alignments were conducted. The sequences for the primer-probe sets were selected from conserved regions within the *B. adriatica* strains, but enabled discrimination from other dinoflagellates. For determinations of optimal melting temperature and secondary structure, the primer and probe sequences of *B. adriatica* were analyzed using Primer 3 (Whitehead Institute, Cambridge, MA, USA; Howard Hughes Medical Institute, Chevy Chase, MD, USA) and Oligo Calc: Oligonucleotide Properties Calculator (Kibbe 2007). Subsequently, the primers and probe were synthesized by BioResearch (Petaluma, CA, USA). The probe was dual-labeled at the 5' and 3' ends with the fluorescent dyes FAM and BHQ1 (BioResearch), respectively (Table 1). A species specificity test for the primers

and probe for *B. adriatica* BATY06 was conducted using nine dinoflagellate species (Appendix 1).

Quantification of the abundance of *Biecheleriopsis adriatica* in Korean waters

For qPCR, 50-300 mL of the water samples collected from each station at each time interval were filtered through a 25-mm GF/C filter (Whatman Inc.). The filter was loosely rolled and placed into a 1.5-mL tube and frozen at -20°C until transfer to the laboratory. For each sample, DNA was extracted from the cells on filters using an *AccuPrep* Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea), according to the manufacturer's instructions.

Standard curve preparation and qPCR amplification

Nucleic acids were extracted from 10 to 15 mL of a dense culture of *B. adriatica* BATY06 as described above. For standard curve construction, DNA was extracted from a dense culture of *B. adriatica* BATY06, containing approximately 1,000,000 cells in the final elution volume of 100 µL, as described above. To prepare seven different DNA concentrations, targeting 100, 10, 1, 0.1, 0.01, 0.001, and 0.0001% of the originally extracted DNA, the extracted DNA was serially diluted by adding predetermined volumes of sterile deionized distilled water (DDW; Bioneer) to the 1.5-mL tubes. Subsequently, the DNA samples were stored at -20°C in a freezer and qPCR amplification was conducted within 24 h. The qPCR assays used for determination of the standard curve were performed using the following steps, as described by Lee et al. (2017b).

Table 1. Oligonucleotide primers used to amplify the SSU, ITS1, 5.8S, ITS2, and LSU regions of ribosomal DNA and the species-specific primers and Taq-Man probe used to determine the abundance of *Biecheleriopsis adriatica* using qPCR

Name	Primer region		5'-3'	Reference
EukA	Forward	SSU	CTGGTTGATCCTGCCAG	Medlin et al. (1988)
G18R	Reverse	SSU	GCATCACAGACCTGTTATTG	Litaker et al. (2003)
G17F	Forward	SSU	ATACCGTCCTAGTCT TAACC	Litaker et al. (2003)
EukB	Reverse	SSU	TGATCCTTCTGCAGGTTACCTAC	Medlin et al. (1988)
ITSF2	Forward	ITS	TACGTCCCCTGCCCTTTGTAC	Litaker et al. (2003)
LSU500R	Reverse	ITS-LSU	CCCTCATGGTACTTGTTTGC	Litaker et al. (2003)
D1RF	Forward	LSU	ACCCGGTGAATTTAAGCA	Scholin et al. (1994)
1483R	Reverse	LSU	GCTACTACCACCAAGATCTGC	Daugbjerg et al. (2000)
Badriatica_F	Forward	ITS	GCAITGTCTTTTGTGTGCCAC	This study
Badriatica_R	Reverse	ITS	CTGTTGCTGATTCACAAGTTGT	This study
Badriatica_P	Probe	ITS	ATTGCTTCGGCTCTGTGGAGCGAGTG	This study

SSU, small subunit; ITS, internal transcribed spacer; LSU, large subunit; qPCR, quantitative real-time polymerase chain reaction.

Fig. 2. Comparison of the morphology of vegetative cells of *Biecheleriopsis adriatica* and other dinoflagellate species observed under a light microscope. Living cells (left column). Cells fixed with 5% Lugol's solution (central column). Cells fixed with 1% formalin solution (right column). (A) *B. adriatica* BATY06 (Ba). (B) *Ansanella granifera* AGSW10 (Ag). (C) *Biecheleria cincta* BCSW0906 (Bc). (D) *Effrenium voratum* SvFL1 (Ev). (E) *Gymnodinium smaydae* GSSW10 (Gs). (F) *Paragymnodinium shiwhaense* PSSH0605 (Ps). (G) *Pelagodinium bei* RCC3655 (Pb). (H) *Pfiesteria piscicida* CCMP2091 (Pp). (I) *Yihiella yeosuensis* YY1405 (Yy). Scale bars represent: A-I, 5 μ m.

Reaction mixtures for each sample were prepared from 1 μL of DNA template, 0.2 μM of primers (forward and reverse), and 0.15 μM of probe (final concentrations), and 5 μL of qPCRBIO Probe Separate-ROX (Genepole, Gwangmyeong, Korea), made up to a final volume of 10 μL with DDW. qPCR analyses were performed using a Rotor-Gene Q thermal cycler (Qiagen, Hilden, Germany) under the following thermal cycling conditions: 3 min at 95°C, followed by 45 cycles of 10 s at 95°C, and 40 s at 58°C.

To determine the concentration of *B. adriatica* in field samples, the aforementioned qPCR assay conditions were used. To ensure the accuracy of results, the DNA extracted from each sample was amplified four times. A sample containing DDW as the template was used as a negative control, whereas one used for standard curve construction was used as positive and standard control.

RESULTS

Comparison of the morphology of nine different dinoflagellates

Morphologically, the living vegetative cells of *B. adriatica* were very similar to those of *A. granifera* (Fig. 2A & B). Furthermore, the overall morphology of *B. adriatica* cells fixed with 5% Lugol's solution was similar to that of *A. granifera*, *B. cincta*, *E. voratum*, *G. smaydae*, *Paragymnodinium shiwhaense*, *Pelagodinium bei*, *Pfiesteria piscicida*, and *Y. yeosuensis* (Fig. 2). Similarly, on the basis of morphology, it was not possible to distinguish *B. adriatica* cells fixed with 1% formalin from the formalin-fixed cells of other species examined in this study (Fig. 2).

Hydrography in the study areas during the study period

From April 2015 to October 2018, the water temperatures at the 28 sampling stations ranged from 0.2 to 28.0°C (Fig. 3A, Appendix 2). The minimum water temperature was recorded in January 2018 at Dangjin and Seocheon (West Sea), whereas the maximum water temperature was recorded at Dangjin in July 2017. Furthermore, the salinity at the 28 stations from April 2015 to October 2018 ranged from 0.1 to 35.6 (Fig. 3A, Appendix 2). The minimum salinity was recorded in March 2016 at Kwangyang (South Sea), and the maximum was recorded at Mageompo (West Sea) in October 2016.

The concentration of NO_3 at the 28 stations from April 2015 to October 2018 ranged from not detectable (ND)

Fig. 3. Environmental parameters recorded at the sampling stations from April 2015 to October 2018. (A) Water temperature (T , °C) versus salinity (S). (B) Nitrate + nitrite (NO_3 , μM) versus phosphate (PO_4 , μM). (C) Silicate (SiO_2 , μM) versus dissolved oxygen (DO , mg L^{-1}).

to 149.0 μM (Fig. 3B, Appendix 3), with the maximum concentration being recorded at Seogwipo (Jeju Island) in July 2015. The concentration of PO_4 at the 28 stations from April 2015 to October 2018 ranged from ND to 6.31 μM (Fig. 3B, Appendix 3), with the maximum concentration being recorded in Jinhae Bay (South Sea) in July 2015. The concentration of SiO_2 at the 28 stations from April 2015 to October 2018 ranged from ND to 453.4 μM (Fig. 3C, Appendix 3), with the maximum concentration being recorded at Seogwipo (Jeju Island) in July 2015. The concentration of DO at the 28 stations from April 2015 to October 2018 ranged from 0.2 to 14.8 mg L^{-1} (Fig. 3C), with maximum concentration being recorded at Ansan (West Sea) in March 2018.

Fig. 4. Map of the sampling stations indicating the presence and absence of vegetative cells of *Biecheleriopsis adriatica* in the spring (March or April) (A) and summer (June or July) (B) during the period from 2015 to 2018. The red closed circles indicate the stations at which *B. adriatica* cells were detected, whereas the black open circles indicate the stations at which *B. adriatica* cells were not detected.

Spatial distribution of *Biecheleriopsis adriatica* in Korean waters

The qPCR analyses indicated the presence of *B. adriatica* in the waters of 20 of the 28 sampling stations (Figs

Fig. 5. Map of the sampling stations indicating the presence and absence of vegetative cells of *Biecheleriopsis adriatica* in the autumn (September or October) (A) and winter (December or January) (B) during the period from 2015 to 2018. The red closed circles indicate the stations at which *B. adriatica* cells were detected, whereas the black open circles indicate the stations at which *B. adriatica* cells were not detected.

4 & 5). The coastal waters in which the species was not detected are as follows: Seochon (West Sea), Kwangyang and Goheung (South Sea), Pohang and Ulsan (East Sea), Seogwipo, Wimi, and Seongsan (Jeju Island). The highest abundance of *B. adriatica* (41.7 cells mL⁻¹) was recorded

Table 2. Mean abundance ($\pm$ standard error, cells mL⁻¹) of the vegetative cells of *Biecheleriopsis adriatica* recorded at each sampling station from April 2015 to December 2016 and also the maximum abundance (Max, cells mL⁻¹) recorded at each sampling station from 2015 to 2018, quantified using quantitative real-time polymerase reactions

Station	Name	2015			2016					2015-2018 Max
		Apr	Jul	Oct	Jan	Mar	Jul	Oct	Dec	
SC	Sokcho	-	-	-	-	-	2.0 (1.0)	-	NA	2.0
JMJ	Jumunjin	NA	3.0 (2.1)	-	-	-	0.1 (0.1)	-	-	4.3
DH	Donghae	-	-	-	-	-	-	-	-	2.4
UJ	Uljin	-	-	-	-	-	-	-	-	-
PH	Pohang	-	-	-	-	-	-	-	-	-
US	Ulsan	-	-	-	-	-	11.1 (1.7)	-	-	11.1
BS	Busan	-	-	-	-	-	1.8 (0.8)	-	-	1.8
DDP	Dadaepo	-	-	-	-	-	2.7 (1.6)	-	-	2.7
JH	Jinhae	NA	-	-	-	-	1.8 (1.5)	9.8 (4.7)	-	41.7
MS	Masan	-	4.0 (2.5)	-	-	-	3.6 (1.7)	-	-	4.0
TY	Tongyoung	-	-	-	-	-	0.4 (0.4)	-	-	0.4
YS	Yeosu	-	-	-	-	-	-	-	-	3.4
KY	Kwangyang	-	-	-	-	-	-	-	-	-
GH	Goheung	NA	-	-	-	-	-	-	-	-
JAH	Jangheung	-	2.6 (1.7)	-	-	-	1.6 (1.0)	-	-	2.6
AS	Ansan	-	-	-	-	-	-	0.1 (0.1)	-	1.4
DAJ	Dangjin	-	-	-	-	-	1.3 (1.3)	-	-	1.3
MGP	Mageompo	-	2.8 (1.1)	-	-	-	2.0 (1.5)	-	-	2.8
TA	Taeon	-	-	0.3 (0.3)	-	NA	1.0 (1.0)	-	-	1.0
SCN	Seocheon	-	-	-	-	-	-	-	-	-
KS	Kunsan	-	3.7 (3.0)	-	-	-	18.3 (5.0)	1.2 (0.8)	-	18.3
BA	Buan	-	2.6 (2.6)	-	-	-	4.0 (1.8)	-	-	4.0
MP	Mokpo	-	-	1.4 (0.9)	-	-	-	-	-	1.4
AW	Aewol	-	-	-	-	-	1.9 (1.3)	4.8 (2.4)	-	4.8
SGP	Seogwipo	-	-	-	-	-	-	-	-	-
WM	Wimi	-	-	-	-	-	-	-	-	-
SS	Seongsan	-	-	-	-	-	-	-	-	-
GN	Gimnyeong	NA	NA	NA	NA	NA	NA	NA	NA	7.0

-, not detected; NA, samples were not available.

Table 3. Mean abundance ($\pm$ standard error, cells mL⁻¹) of the vegetative cells of *Biecheleriopsis adriatica* recorded at each sampling station from March 2017 to October 2018 and also the maximum abundance (Max, cells mL⁻¹) recorded at each sampling station from 2015 to 2018, quantified using quantitative real-time polymerase reactions

Station	Name	2017			2018				2015-2018 Max
		Mar	Jul	Oct	Jan	Mar	Jul	Oct	
SC	Sokcho	-	0.1 (0.1)	-	-	-	-	-	2.0
JMJ	Jumunjin	-	4.3 (1.9)	-	-	-	2.1 (1.3)	-	4.3
DH	Donghae	-	2.4 (1.0)	-	-	-	0.2 (0.2)	-	2.4
UJ	Uljin	-	-	-	-	-	-	-	-
PH	Pohang	-	-	-	-	-	-	-	-
US	Ulsan	-	-	-	-	-	-	-	11.1
BS	Busan	-	-	-	-	-	-	-	1.8
DDP	Dadaepo	-	0.8 (0.8)	-	-	-	-	-	2.7
JH	Jinhae	-	25.3 (7.9)	-	-	-	41.7 (9.3)	-	41.7
MS	Masan	-	-	-	-	-	-	-	4.0
TY	Tongyoung	-	-	-	-	-	-	-	0.4
YS	Yeosu	-	3.4 (1.2)	-	-	-	-	-	3.4
KY	Kwangyang	-	-	-	-	-	-	-	-
GH	Goheung	-	-	-	-	-	-	-	-
JAH	Jangheung	-	1.0 (0.5)	-	-	-	-	-	2.6
AS	Ansan	-	1.4 (0.9)	-	-	-	NA	-	1.4
DAJ	Dangjin	-	-	-	-	-	-	-	1.3
MGP	Mageompo	-	-	-	-	-	-	-	2.8
TA	Taeon	NA	NA	NA	NA	NA	NA	NA	1.0
SCN	Seocheon	-	-	-	-	-	-	-	-
KS	Kunsan	-	-	-	-	-	-	-	18.3
BA	Buan	-	-	-	-	-	0.9 (0.8)	-	4.0
MP	Mokpo	-	-	-	-	-	-	-	1.4
AW	Aewol	-	-	-	-	-	-	-	4.8
SGP	Seogwipo	-	-	-	-	-	-	-	-
WM	Wimi	-	-	-	-	-	-	-	-
SS	Seongsan	-	-	-	-	-	-	-	-
GN	Gimnyeong	-	7.0 (2.1)	-	-	-	-	-	7.0

-, not detected; NA, samples were not available.

in Jinhae Bay (South Sea) in July 2018. The second highest abundance (25.3 cells mL⁻¹) was also recorded in Jinhae Bay in July 2017, whereas the third highest abundance, (18.3 cells mL⁻¹) was recorded at Kunsan (West Sea) in July 2016 (Tables 2 & 3).

Seasonal distribution of *Biecheleriopsis adriatica* in Korean waters

We found that cells of *B. adriatica* were only present in samples collected in the summer and autumn months (Figs 4 & 5). Moreover, the number of stations at which *B. adriatica* cells were detected in the summer (19) was considerably greater than that at which they were detected in the autumn (6) (Figs 4 & 5). Furthermore, the maximum abundance of *B. adriatica* recorded in the summer (41.7 cells mL⁻¹) was considerably higher than that recorded in the autumn (9.8 cells mL⁻¹). Thus, these results provide evidence of a strong seasonality in the occurrence and abundance of *B. adriatica* in Korean waters.

The summer water temperatures and salinities at stations where *B. adriatica* was detected ranged from 17.7 to 26.4°C and 9.9 to 33.8, respectively, whereas those in the autumn ranged from 19.4 to 23.5°C and 24.6 to 34.3, respectively (Fig. 6A & B, Appendix 2). Furthermore, the concentrations of NO₃, PO₄, SiO₂, and DO in the summer at the stations where *B. adriatica* was detected ranged from ND to 96.2, 0.18 to 2.66, and 2.4 to 448.4 µM, and 3.7 to 10.8 mg L⁻¹, respectively, whereas those in the autumn ranged from 4.4 to 25.0, 0.34 to 1.78, and 38.4 to 132.7 µM, and 6.1 to 7.9 mg L⁻¹, respectively (Fig. 6C-F, Appendix 3).

The water temperatures and salinities coinciding with the highest, second highest, and third highest recorded abundances of *B. adriatica* in Jinhae Bay, and at Kunsan ranged from 23.2 to 23.8°C and 22.8 to 29.3, respectively, whereas the concentrations of NO₃, PO₄, SiO₂, and DO ranged from 8.7 to 28.8, 0.44 to 1.65, and 36.4 to 70.3 µM, and 5.4 to 10.8 mg L⁻¹, respectively (Fig. 6).

Correlations between *Biecheleriopsis adriatica* abundance and environmental parameters

Analyses of all data obtained from all stations and all sampling times (n = 395) revealed that abundances of the vegetative cells of *B. adriatica* were significantly correlated with water temperature and salinity ($p < 0.01$ or 0.05), but was not significantly correlated with NO₃, PO₄, SiO₂, or DO concentrations ($p > 0.1$) (Table 4). However, we did find that water temperature was significantly positively correlated with NO₃, PO₄, and SiO₂ concentrations, but

Fig. 6. Abundance (Ab, cells mL⁻¹) of the vegetative cells of *Biecheleriopsis adriatica* as a function of each of the recorded environmental parameters at all sampling stations from April 2015 to October 2018 (n = 395). (A) Water temperature (T, °C). (B) Salinity (S). (C) Nitrate + nitrite (NO₃, µM). (D) Phosphate (PO₄, µM). (E) Silicate (SiO₂, µM). (F) Dissolved oxygen (DO, mg L⁻¹). The red closed circles indicate the stations at which *B. adriatica* cells were detected, whereas the black open circles indicate the stations at which *B. adriatica* cells were not detected.

negatively with salinity and DO concentration ($p < 0.01$) (Table 4). Moreover, salinity was significantly positively correlated with DO, but negatively correlated with NO₃, PO₄, and SiO₂ concentrations ($p < 0.01$) (Table 4), whereas NO₃ concentration was significantly positively correlated with PO₄ and SiO₂ concentrations ($p < 0.01$) (Table 4).

In Jinhae Bay, in which we recorded the maximum abundance of the vegetative cells of *B. adriatica*, vegetative cell abundance was significantly positively correlated with the concentration of NO₃ ($p < 0.01$), but

Table 4. Pearson's correlation coefficients for relationships between abundance of the vegetative cells of *Biecheleriopsis adriatica* (Ba, cells mL⁻¹) and temperature (T, °C), salinity (S), and the concentrations of nitrate + nitrite (NO₃, µM), phosphate (PO₄, µM), silicate (SiO₂, µM), and dissolved oxygen (DO, mg L⁻¹) in water samples collected at each station from April 2015 to October 2018

	Ba	T	S	NO ₃	PO ₄	SiO ₂	DO
Ba	-	0.167**	-0.129*	0.043	-0.004	0.046	-0.011
T	0.167**	-	-0.354**	0.303**	0.159**	0.348**	-0.614**
S	-0.129*	-0.354**	-	-0.666**	-0.219**	-0.626**	0.217**
NO ₃	0.043	0.303**	-0.666**	-	0.330**	0.826**	-0.167**
PO ₄	-0.004	0.159**	-0.219**	0.330**	-	0.300**	-0.311**
SiO ₂	0.046	0.348**	-0.626**	0.826**	0.300**	-	-0.216**
DO	-0.011	-0.614**	0.217**	-0.167**	-0.311**	-0.216**	-

*p < 0.05, **p < 0.01.

Table 5. Pearson's correlation coefficients for relationships between abundance of the vegetative cells of *Biecheleriopsis adriatica* (Ba, cells mL⁻¹) and temperature (T, °C), salinity (S), and the concentrations of nitrate + nitrite (NO₃, µM), phosphate (PO₄, µM), silicate (SiO₂, µM), and dissolved oxygen (DO, mg L⁻¹) in water samples collected in Jinhae Bay from April 2015 to October 2018

	Ba	T	S	NO ₃	PO ₄	SiO ₂	DO
Ba	-	0.480	-0.835**	0.729**	-0.072	0.438	0.082
T	0.480	-	-0.628*	0.559*	0.349	0.508	-0.495
S	-0.835**	-0.628*	-	-0.943**	-0.081	-0.690**	0.164
NO ₃	0.729**	0.559*	-0.943**	-	0.177	0.745**	-0.260
PO ₄	-0.072	0.349	-0.081	0.177	-	0.204	-0.174
SiO ₂	0.438	0.508	-0.690**	0.745**	0.204	-	-0.469
DO	0.082	-0.495	0.164	-0.260	-0.174	-0.469	-

*p < 0.05, **p < 0.01.

Table 6. Worldwide distribution of the vegetative cells and cysts of *Biecheleriopsis adriatica*

Form / Region	Date	Latitude	Temperature (°C)	Salinity	Reference
Vegetative cells					
East, West, and South Sea, Korea	Apr 2014-Oct 2018	33°17' N-38°12' N	17.7-26.4	9.9-34.3	This study
Lake Hamana, Japan	Jun 2014	34°41' N	21.0	35.0	Benico et al. (2019)
Matsushima Bay, Japan	Jul 2013	38°16' N	22.0	28.0	Benico et al. (2019)
Pohang, Korea	Sep 2012	36°10' N	23.0	29.6	Jang et al. (2015)
Nabeta Bay, Japan	Oct 2010	34°36' N	24.0	NA	Takahashi et al. (2014)
Shiwha Bay, Korea	Sep 2009	37°33' N	25.1	24.0	Jang et al. (2015)
Tongyoung, Korea	Aug 2006	35°04' N	28.0	31.0	Jang et al. (2015)
Sado Island, Japan	Sep 2012	38°04' N	28.4	NA	Takahashi et al. (2014)
Sendai Haror, Japan	Aug 2010	38°27' N	29.0	NA	Takahashi et al. (2014)
Pacific, Palau Island	Nov 2013	07°14' N	28.5	34.0	Benico et al. (2019)
Bolinao Channel, Philippines	May 2016	16°21' N	31.0	30.0	Benico et al. (2019)
Bolinao Channel, Philippines	May 2017	16°20' N	32.0	30.0	Benico et al. (2019)
Tsuruoka, Japan	Jul, Aug 2013	38°45' N	NA	NA	Benico et al. (2019)
St. John's Island, Singapore	Jul 2016	01°13' N	NA	30.0	Benico et al. (2019)
Adriatic Sea	NA	NA	NA	NA	Moestrup et al. (2009)
Yellow Sea, China	Apr 2012	34°44' N	NA	NA	Luo et al. (2015)
Cysts					
Zhelin Bay, China	Nov 2008	NA	NA	NA	Kang and Wang (2018)
Guishan Island, China	Jan 2009	NA	NA	NA	Kang and Wang (2018)
Yellow Sea, China	Apr 2011	39°15' N	NA	NA	Luo et al. (2015)
Yellow Sea, China	May 2011	34°48' N	NA	NA	Luo et al. (2015)
South China Sea	May 2010	21°29' N	NA	NA	Luo et al. (2015)
South China Sea	Jun, Jul 2011	21°29' N	NA	NA	Luo et al. (2015)
South China Sea	Jun 2012	21°29' N	NA	NA	Luo et al. (2015)
Black Sea	NA	NA	NA	NA	Dzhembekova et al. (2018)

NA, not available.

negatively correlated with salinity ($p < 0.01$) (Table 5, Fig. 7). Furthermore, water temperature was significantly positively correlated with NO_3 concentration ($p < 0.05$), but negatively correlated with salinity ($p < 0.05$) (Table 5). Moreover, salinity was significantly negatively with NO_3 and SiO_2 concentrations ($p < 0.01$), but showed no significant correlation with PO_4 concentration ($p > 0.1$) (Table 5). Similarly, NO_3 concentration was significantly positively correlated with SiO_2 concentration ($p < 0.01$), but showed no significant correlation with PO_4 concentration ($p > 0.1$) (Table 5).

DISCUSSION

The results of the present study indicate that, morphologically, the vegetative cells of *B. adriatica* cannot be distinguished from those of several other dinoflagellates when the cells are fixed with either 5% Lugol's solution or 1% formalin and then observed under a light microscope. Accordingly, difficulties are likely to be encountered in attempting to quantify the abundance of *B. adriatica* in fixed field samples. In contrast, qPCR would appear to be an acceptable method for quantifying *B. adriatica* abundance.

We found that the vegetative cells of *B. adriatica* have a wide spatial distribution in Korean waters. Previously, the vegetative cells of *B. adriatica* have been collected from the waters off Tongyoung, Pohang, and Shihwa, which are located between the latitude of $35^{\circ}04' \text{ N}$ and $37^{\circ}33' \text{ N}$ (Jang et al. 2015). In the present study, we found that the latitudinal range within which vegetative cells of *B. adriatica* were collected extends from $33^{\circ}17' \text{ N}$ to $38^{\circ}12' \text{ N}$. Thus, the findings of this study widen the latitudinal range in which vegetative cells of *B. adriatica* are known to occur in Korean waters. Furthermore, prior to the present study, the global distribution of the vegetative cells of *B. adriatica* was reported to lie within the latitudes $01^{\circ}13' \text{ N}$ to $38^{\circ}45' \text{ N}$ (Table 6). Thus, our observations do not extend the currently established latitudinal range of the vegetative cells of this dinoflagellate.

We also discovered that the occurrence of vegetative *B. adriatica* cells shows a strong seasonality. Vegetative cells were recorded from July and October when water temperatures were between 17.7 and 26.4°C . Previously, in Korea, vegetative cells of *B. adriatica* have been recorded in August and September when recorded water temperatures were between 23.0 and 28.0°C (Jang et al. 2015). Thus, on the basis of the findings of the present study, we have established that in Korean waters, the tempera-

Fig. 7. Abundance (Ab, cells mL⁻¹) of the vegetative cells of *Biecheleriopsis adriatica* as a function of each of the recorded environmental parameters in Jinhae Bay from April 2015 to October 2018 ($n = 14$). (A) Water temperature (T, °C). (B) Salinity (S). (C) Nitrate + nitrite (NO_3 , μM). (D) Phosphate (PO_4 , μM). (E) Silicate (SiO_2 , μM). (F) Dissolved oxygen (DO, mg L⁻¹). The p-value in (B) and (C) is < 0.01 , whereas that in (A), (D), (E), and (F) is > 0.1 . The equations of the curves are as follows: $\text{Ab (cells mL}^{-1}\text{)} = -3.29 (\text{S}) + 106$, and $r^2 = 0.697$ for (B) and $\text{Ab (cells mL}^{-1}\text{)} = 1.07 (\text{NO}_3) - 4.83$, and $r^2 = 0.531$ for (C).

tures at which vegetative cells of *B. adriatica* occur ranges from 17.7 to 28.0°C . Given that, prior to the present study, the occurrence of vegetative *B. adriatica* cells in oceans worldwide has been reported in waters with temperatures ranging from 21.0 to 32.0°C (Table 6), the findings of the present study extends this range to 17.7 – 32.0°C .

In terms of salinity, we found that the vegetative cells of *B. adriatica* occur within the range 9.9 – 34.3 . At the lowest recorded salinity, the abundance of the vegetative cells of *B. adriatica* was 3.6 cells mL⁻¹. Thus, this abundance is not a negligible value. Previously, vegetative cells of *B. adriatica* were found in Korean waters with salinities ranging from 24.0 to 31.0 (Jang et al. 2015). Thus, on the basis of the findings of the present study, we have estab-

lished an extended range of salinities (9.9-34.3) in which the vegetative cells of *B. adriatica* are present in Korean waters. Furthermore, given that the previously reported range for oceans worldwide was 24.0-35.0, our findings also extend the global range of salinities in which the vegetative cells of *B. adriatica* have been reported to 9.9-35.0 (Table 6).

Prior to the present study, there had been no studies reporting data on the concentrations of NO_3 , PO_4 , and SiO_2 in waters inhabited by the vegetative cells of *B. adriatica*. Thus, relationships between the concentrations of NO_3 , PO_4 , and SiO_2 and the presence of these vegetative cells are reported here for the first time. We found that the vegetative cells of *B. adriatica* occur in Korean water with wide-ranging concentrations of NO_3 , PO_4 , and SiO_2 (ND-96.2, 0.18-2.66, and 2.4-448.4 μM , respectively). However, when all the data were analyzed, it was apparent that vegetative cell abundance showed no significant correlation with the concentrations NO_3 , PO_4 , SiO_2 , and DO, and thus it seems unlikely that these parameters have any pronounced effect on the presence and abundance of the vegetative cells of *B. adriatica* in Korean waters. Having said this, however, in the waters Jinhae Bay, we did find at least some evidence of a relationship between vegetative cell abundance and nutrient concentrations. It was in these waters in which we recorded the maximum abundance of *B. adriatica* vegetative cells, and the concentrations of NO_3 , PO_4 , and SiO_2 were 28.8, 0.44, and 70.3 μM , respectively. Given that the ranges of NO_3 , PO_4 , and SiO_2 concentrations recorded in Jinhae Bay during the study period were 1.1-28.8, 0.22-6.31, and 4.5-84.6 μM , respectively, it can be seen that the vegetative cells of *B. adriatica* were most abundant when the concentration of NO_3 was at its highest level. Indeed, abundances of the vegetative cells of *B. adriatica* were found to be significantly positively correlated with the concentration of NO_3 in the waters of Jinhae Bay. Thus, the concentration of NO_3 may be a critical factor affecting the abundance of the vegetative cells of *B. adriatica* in this bay.

Interestingly, however, the maximum abundance of the vegetative cells of *B. adriatica* in Korean waters recorded in the present study (41.7 cells mL^{-1}) is somewhat lower than that recorded at Bolinao in the Philippines (299-305 cells mL^{-1}) (Benico et al. 2019), whereas the mean concentration of NO_3 at Bolinao, during the period from 1995 to 2005 was only 0.21-2.17 μM (San Diego-McGlone et al. 2008), which is considerably lower than the concentration recorded in Jinhae Bay during the present study period. Thus, it is conceivable that the ecophysiology of *B. adriatica* in Jinhae Bay differs from that of *B. adriatica* in

the waters off Bolinao. However, further studies will be necessary to verify this conjecture.

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Appendix 1. List of dinoflagellate species used to determine primer specificity

Species	Strain name	Location of collection	Accession number (ITS)
<i>Biecheleriopsis adriatica</i>	BATY06	Tongyoung, Korea	LM992906
<i>Biecheleriopsis adriatica</i>	BAPH12	Pohang, Korea	LM992904
<i>Biecheleriopsis adriatica</i>	BASW09 (= BASH09)	Shiwha, Korea	LM992905
<i>Ansanella granifera</i>	AGSW10	Shiwha, Korea	HG529979
<i>Biecheleria cincta</i>	BCSW0906	Shiwha, Korea	FR690459
<i>Gymnodinium smaydae</i>	GSSW10	Shiwha, Korea	HG005135
<i>Paragymnodinium shiwhaense</i>	PSSH0605	Shiwha, Korea	AM408889
<i>Pelagodinium bei</i>	HJ-2010	Shiwha, Korea	KP342301
<i>Effrenium voratum</i>	SvFL 1	Jeju, Korea	HF568830
<i>Yihiella yeosuensis</i>	YY1405	Yeosu, Korea	LN898222
<i>Luciella masanensis</i>	LMJH1607 (Masan Lucy200505)	Jinhae, Korea	AM050344
<i>Pfiesteria piscicida</i>	CCMP2091	Neuse River, USA	DQ344043

ITS, internal transcribed spacer.

Appendix 2. The range of temperatures and salinities recorded from April 2015 to October 2018 at all sampling stations at which water samples were collected (ASW), and at the stations at which vegetative cells of *Biecheleriopsis adriatica* were detected (SBD)

Station	Name	Temperature (°C)		Salinity	
		ASW	SBD	ASW	SBD
SC	Sokcho	5.2-21.6	19.7-21.6	31.2-34.3	33.0-33.4
JMJ	Jumunjin	5.4-21.2	18.9-21.2	30.2-33.8	30.3-33.7
DH	Donghae	6.8-22.9	19.7-22.5	30.1-34.2	30.5-33.1
UJ	Uljin	9.2-22.9	-	31.2-34.6	-
PH	Pohang	9.2-23.5	-	22.0-34.4	-
US	Ulsan	10.2-23.8	19.4	30.2-34.4	33.8
BS	Busan	10.5-23.5	17.7	23.2-34.3	32.5
DDP	Dadaepo	7.3-23.1	19.4-21.4	26.3-34.2	26.3-32.3
JH	Jinhae	5.9-23.8	22.4-23.8	22.8-33.6	22.8-30.7
MS	Masan	5.6-24.0	22.3-24.0	9.9-33.0	9.9-25.3
TY	Tongyoung	1.1-22.3	22.0	21.8-33.4	32.8
YS	Yeosu	4.4-26.4	26.4	27.1-33.6	30.1
KY	Kwangyang	7.4-27.1	-	0.1-33.2	-
GH	Goheung	2.3-26.1	-	19.4-34.0	-
JAH	Jangheung	3.8-24.7	23.0-24.3	26.4-33.1	26.4-31.0
AS	Ansan	1.7-25.6	19.8-25.6	21.1-34.3	30.3-34.3
DAJ	Dangjin	0.2-28.0	24.9	24.7-32.5	24.7
MGP	Mageompo	1.9-23.6	23.0-23.6	31.0-35.6	31.5-31.6
TA	Taeon	6.0-24.1	19.4-23.9	28.8-35.1	28.8-32.8
SCN	Seocheon	0.2-27.0	-	19.5-34.5	-
KS	Kunsan	1.8-23.8	21.8-23.2	29.3-33.9	29.3-33.8
BA	Buan	2.2-25.0	23.9-24.8	11.5-34.1	22.8-29.4
MP	Mokpo	5.0-25.8	21.5	9.6-33.6	30.7
AW	Aewol	12.5-24.3	20.0-23.5	22.7-33.1	23.6-26.7
SGP	Seogwipo	14.8-24.2	-	17.5-33.1	-
WM	Wimi	14.0-23.6	-	20.8-33.8	-
SS	Seongsan	11.5-25.5	-	29.9-34.3	-
GN	Gimnyeong	10.8-24.5	24.5	20.0-31.9	20.0
	Total	0.2-28.0	17.7-26.4	0.1-35.6	9.9-34.3

Appendix 3. The range of nitrite + nitrate (NO₃), phosphate (PO₄), and silicate (SiO₂) concentrations recorded from April 2015 to October 2018 at all sampling stations at which water samples were collected (ASW), and at the stations at which the vegetative cells of *Biecheleriopsis adriatica* were detected (SBD)

Station	Name	NO ₃ (μM)		PO ₄ (μM)		SiO ₂ (μM)	
		ASW	SBD	ASW	SBD	ASW	SBD
SC	Sokcho	0.2-6.8	0.2-1.4	0.03-6.06	0.20-0.26	0.6-24.7	2.4-14.1
JMJ	Jumunjin	1.0-11.5	1.0-6.5	0.18-2.17	0.18-0.69	1.9-59.7	6.6-29.3
DH	Donghae	2.0-14.2	4.9-6.6	0.10-0.98	0.20-0.27	6.1-51.1	16.3-20.8
UJ	Uljin	0.8-43.6	-	0.14-1.39	-	2.7-35.2	-
PH	Pohang	0.9-63.0	-	0.03-0.80	-	10.8-232.1	-
US	Ulsan	ND-10.8	ND	0.15-1.37	0.21	4.3-38.2	4.3
BS	Busan	2.8-26.5	2.8	0.39-1.87	0.59	13.7-140.4	23.0
DDP	Dadaepo	2.9-29.2	7.4-29.2	0.05-4.22	0.72-0.93	3.5-64.4	14.0-44.8
JH	Jinhae	1.1-28.8	6.4-28.8	0.22-6.31	0.44-1.65	4.5-84.6	23.3-71.6
MS	Masan	0.7-91.9	18.7-91.9	0.17-3.38	0.72-2.66	4.1-316.6	57.4-316.6
TY	Tongyoung	0.4-42.0	17.6	0.01-2.83	1.10	5.4-178.4	63.1
YS	Yeosu	0.8-26.4	2.9	0.19-1.13	0.19	ND-64.0	29.3
KY	Kwangyang	2.7-102.9	-	0.08-3.45	-	6.6-243.2	-
GH	Goheung	0.9-34.6	-	0.18-1.34	-	7.4-105.9	-
JAH	Jangheung	0.4-17.5	5.2-12.6	ND-1.02	0.52-0.83	0.0-81.1	44.4-65.8
AS	Ansan	2.8-22.7	3.3-20.0	ND-1.78	0.19-1.78	0.6-65.9	20.6-48.6
DAJ	Dangjin	12.1-42.7	31.4	0.01-2.18	1.76-1.76	1.2-83.3	67.0
MGP	Mageompo	0.1-10.7	1.7-5.0	ND-0.93	0.44-0.86	ND-41.6	32.0-37.2
TA	Taeon	1.6-10.7	4.4-8.0	0.14-1.66	1.43-1.66	1.2-82.7	54.7-82.8
SCN	Seocheon	0.7-42.4	-	0.01-1.99	-	3.1-117.7	-
KS	Kunsan	1.0-41.4	3.3-8.7	ND-1.66	0.52-1.19	ND-53.6	31.3-50.5
BA	Buan	1.2-118.3	13.9-44.7	0.24-4.67	1.06-1.47	2.6-245.2	62.7-130.9
MP	Mokpo	2.4-106.0	14.2	0.14-6.26	1.14	2.0-236.3	38.4
AW	Aewol	8.0-96.2	24.7-96.2	0.18-2.66	0.34-2.66	30.6-273.0	132.7-273.0
SGP	Seogwipo	10.2-149.0	-	0.21-3.59	-	21.5-453.4	-
WM	Wimi	8.2-82.8	-	0.41-1.09	-	19.2-279.3	-
SS	Seongsan	4.5-86.4	-	0.27-0.92	-	16.7-109.1	-
GN	Gimnyeong	9.1-89.0	59.6	0.46-1.50	1.23	40.9-448.4	448.4
	Total	ND-149.0	ND-96.2	ND-6.31	0.18-2.66	ND-453.4	2.4-448.4

ND, concentration was not detectable; -, cell was not detected.