Thesis for the Degree of Master of Fisheries Science

Bloom Potential of Toxic Dinoflagellate *Alexandrium* spp. in Jinhae Bay, Korea

by

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한국의 진해만 해역에서 발생하는 유해성 와편모조류 *Alexandrium* spp.의 발생잠재력 조사

Advisor: Prof. Chang-Hoon Kim

by

Ibrahim Musa Abdelwahab Ahamed

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Abstract

Harmful algal blooms (HABs) are phenomenon which shows serious impacts on the marine ecosystem and aquaculture productivity in many areas of the world. In Korea, Jinhae Bay is a highly developed coastal area in aquaculture productivity, where mussels, oysters and fish farms are widely distributed. however, the occurrences of HABs and paralytic shellfish poisoning (PSP) caused by the genus *Alexandrium* which has frequently bloomed around Jinhae Bay are imposing serious damage to public health as well as shellfish farming.

To examine bloom potential and development of the toxic dinoflagellate *Alexandrium* spp., 182 sediment samples collected from 14 stations in Jinhae Bay from December 2011 to December 2012 by National Fisheries Research and Development Institute (NFRDI) were analysed, and the occurrence of vegetative cells and abundance of resting cysts of

Alexandrium spp. at two periodic stations (CCSt. 1 and CCSt. 2) in Jinhae Bay were monthly surveyed from from February to May 2013.

Water temperature (°C) averaged on the periodic sampling stations in Jinhae Bay from Dec 2011 to Dec 2012 shows a clear seasonal variation pattern with lower ranges in winter and higher in summer. Also on the physical factors such as pH and DO (mg/L) observed at the stations near Chilcheon Island in Jinhae Bay from Feb to May 2013 indicated for the highest mean in the summer season and the lowest in the winter, while the salinity ranges high in winter and low during spring season.

Alexandrium spp. cysts were found in 182 sediment samples received from National Fisheries Research and Development Institute (NFRDI) at 14 stations in Jinhae Bay from Dec 2011 to Dec 2012, but the abundance was highly variable in a concentration range of 14-250 cysts/cm^3 by showing spatio-temporal distribution patterns. Also the abundance of *Alexandrium* cysts varied horizontally among the sampling stations, where the highest concentration was found in St.11 (141.5±51.9 cysts/ cm^3) and relatively higher distribution pattern showed around Jindong Bay stations.

When a relationship between motile cells occurrence and cysts abundance was focused on the periodic stations near Chilcheon Island during vernal warming period, vegetative cells and cysts of *Alexandrium* spp. were also present at two stations of CCSt. 1 and CCSt. 2. moreover, there was a close relationship between the occurrence of vegetative cells and the abundance of cysts. vegetative cells of *Alexandrium* spp. appeared at all the stations in February, and reached maximum concentration (13.6±2.8 cells/L) in April. In contrast, *Alexandrium* cysts showed in maximum abundance in February, and the highest concentration of vegetative cells was followed by a sharp decrease in the cysts abundance, adversely both with temperature elevation and with the increase of motile cells.

Motile cells recurrent in the cold season could result from the germination of their cysts which have been controlled largely by the environmental and biological regimes. Considering the extensive occurrence of toxic algal species with the same seasonality as a cysts germination, it is suggested that monitoring for the prediction of regional impacts should be undertaken based on the life cycle strategies of causative organisms.

1. Introduction

1.1. Background

Some algae are capable of producing powerful toxins that are harmful to other species, may include fish kills, injuries to marine invertebrates, and human illness or death (Matrai et al., 2005). over 100 species of dinoflagellates are capable of forming harmful algal blooms (HABs), of which 50 species are known to be toxic. In addition, the genus *Alexandrium* includes 28 species, of which described 8 species are known to cause paralytic shellfish poisoning (PSP), while the others are non-toxic species. moreover, harmful algal blooms produce natural toxins and various types of paralytic shellfish poisoning (PSP) that causes negative impacts with large-scale of marine ecosystem (Gracia et al., 2013). associated with PSP occurrence in coastal waters throughout the world, from northern to tropical latitudes, some of these toxic species produce varying amounts and combinations of potent neurotoxins such as saxitoxins and gonyautoxins, leading to highly variable intrinsic potencies (Martins et al., 2004).

Therefore, many researchers has been focused to understanding the biological, physical and chemical factors that control initiation, maintenance and cells function of variety of HAB-forming species which often produce potent toxins cause contaminants in aquaculture environment (Figueroa et al., 2008). To prevent problems caused by PSP, it is necessary to study and enumerate these toxic species before and during a bloom (Nagai et al., 2012; Lacasse et al., 2013; Sournia, 1995).

The sexual reproduction of the genus *Alexandrium* includes a dormant hypnozygote cyst formation, which is fall to the bottom where they undergo a mandatory period of quiescence at inappropriate condition (Kirn et al., 2005). In addition, only about 15% of them will produce hypnozygote (Anderson et al., 2012). A thick protective wall improves the survival of resting cysts and facilitates their sinking to the sea bottom. moreover, it can rest in the sediments for decades, consequently survive long as of potential diversity (Angles et al., 2010). cysts germination of *Alexandrium* spp. has been affected by both environmental and physiological factors such as temperature, light, salinity, turbulence, and time of year (Kirn et al., 2005), as well as biological factors such as algal-bacterial interactions are increasingly cited as potential regulators both in the sense of decreasing and enhancing algal blooming (Su et al., 2007). furthermore, marine microalgae blooming in coastal waters has been indicated to the absorption of nutrients including iron, which is very importance for microalgae as it acts to cysts formation structure (Subba Rao and Stewart, 2011). in fact, the cysts are very important for species dispersal, bloom initiation and termination in bottom sediments, and overlying winters when conditions are unsuitable for growth (Anderson, 1978; Anderson et al., 2005). accordingly, examination of the factors influencing the distribution, density and germination ability of resting cysts is important in order to clarify the mechanism of blooms by these toxic dinoflagellates (Tsujino et al., 2002).

Dinoflagellate cysts range in size from 10 to 100 ㎛, and thus they behave as fine silt particles in the natural environment. therefore, they can be easily transported by surface and bottom currents (Goodman, 1987). Resting cysts are mainly distributed at the sediment surface where their concentration increases in muddy sediment with water and organic matter contents. however, the observed variations in resting cysts distribution and density could be linked to the encystment capability of planktonic species in the water column, sedimentation, transport and bioturbation processes. mud sediment combined with high sedimentation rate promotes resting cysts burying and resuspension can affect their distribution. moreover, bioturbation contributes to their vertical redistribution into the sediment. all these processes result in heterogeneous distribution of resting cysts (Genovesi et al., 2007).

Jinhae Bay, a semi-enclosed and often the water depth ranging between 5 to 20m, affected with coastal tide, located in the south eastern part of Korea, include many island and small bays such as Masan Bay, Haengam Bay, Jindong Bay, Gohyun Bay (Park et al., 1995; Lee, 1998). Jinhae Bay includes mariculture such as bivalves and finfish and aquaculture for oyster, sea squirt and ark clam which is the most popular fisheries industry around the Bay area (Lee et al., 2008). however, the harmful algal blooms (HABs) including the toxigenic dinoflagellate the *Alexandrium tamarense/ catenella* (Dinophyceae) have frequently occurred at Jinhae Bay, and recently are increasing in intensity and spreading to new areas. especially, toxic *Alexandrium* species, largely *A. tamarense,* causing PSP intoxification, have occurred during spring and or autumn season almost every year in shellfish culture farm not only in Jinhae Bay but also in the western coast of Busan, these are harvesting sites for shellfish such as oysters and mussel harvest has been frequently banned mainly from March to April based on the PSP toxin monitoring results (Han et al., 1993; Kim and Shin, 1997).

Considering the extensive occurrence of harmful and toxic algal species showing a seasonal period, the development of monitoring techniques, particularly, based on life cycle would be beneficial for the early prediction and warning of HAB occurrence or intoxification of bivalves.

1.2. *Alexandrium* life cycle stages

The life cycle of *Alexandrium* has been described by Dale (1977), Anderson and Wall (1978) (Fig. 1). this life cycle includes a motile vegetative cell that divides mitotically, a temporary resting cyst or pellicle cyst, motile anisogamous gametes, a motile planozygote that develops into a dormant hypnozygote cyst, and a motile planomeiocyte, the stage that emerges from the cyst.

Vegetative cells or motile cells as they are sometimes called, are roughly 36 μm in diameter with two flagellate, one transverse and the other one longitudinal (Dale, 1977). vegetative cells can swim on the order of 10 m per day (Kirn et al.,2005).

Pellicle cysts during times of environmental stress, vegetative cells can shed their thecae and become pellicle, or temporary cysts - athecate non motile cells of spherical or ovoid shapes (Anderson and Wall., 1978). internal structures can either resemble those of motile cells or hypnozygotes is capable of surviving for one to seven months in this temporary stage while environmental conditions are unsuitable before they must resume the vegetative cell stage or die. most pellicle cysts are not viable after only two months (Anderson and Wall., 1978).

Hypnozygote cysts are usually capsule shaped and 45-55 μm by 25-30 μm, although shape and size vary somewhat (Anderson, 1980). hypnozygote cysts have a mandatory dormancy period on the order of 2-6 months; there is evidence that the length of dormancy is temperature dependent, with excystment being possible earlier in cysts incubated at warmer temperatures (Anderson, 1980). cysts can survive burial in sediments for many years and still germinate successfully (Keafer et al., 1992).

Planomeiocytes are also called germLings or germLing cells. they are posteriorly biflagellate, diploid, and at 40-50 pm, larger than vegetative cells soon after excystment, the planomeiocytes undergo their first division within 24 hours (Anderson and Wall, 1978), within 12-36 hours for a variety of dinoflagellate species (Nehring, 1996).

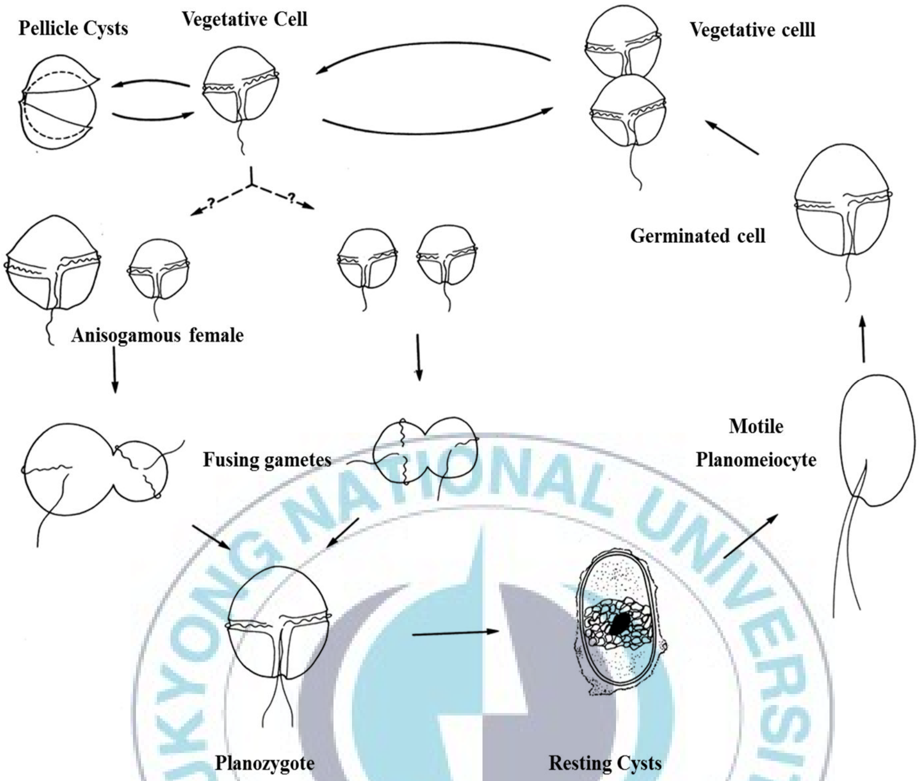

Fig. 1. Life cycle diagram of *Alexandrium* spp. (modified from Anderson, 1998).

1.3. Hypothesis

Some species of flagellates form resting cysts, which settle on the sea floor and can remain there for years. the important role of cysts to the bloom initiation has been well pointed out (Anderson and Wall, 1978; Anderson et al., 1983), because the timing and location of algal blooms can depend on when the cysts germinate and where they were deposited, respectively.

The bloom potential and germination rates within the *Alexandrium* spp. in Jinhae Bay has been changing with the variation of environmental factors such as temperature, pH, salinity, nutrients, and seasonal regimes.

1.4. Study objectives

Studies on the sediments analysis have also been conducted to document the historical occurrence of harmful dinoflagellate. however, in Korea coastal areas, the distribution of *Alexandrium* cysts remains poorly documented, their relations to environmental conditions are not fully understood, and environmental conditions of dinoflagellate cyst assemblages has not been attempted (Shin et al*.*, 2010). besides few studies on the bloom potential of toxic dinoflagellate of *Alexandrium* spp. have been done. this study aims to:

I. To know how *Alexandrium* spp. develop to impact on the mussels and aquaculture industry in Jinhae Bay.

II. To compare and determine the growth and bloom potential of *Alexandrium* spp. during the different seasons and environmental factors.

2. Materials and methods

2.1. Study site

Sediment samples were collected by National Fisheries Research and Development Institute (NFRDI) from fourteen stations (Stns. 2-15) on the periodic monitoring stations in Jinhae Bay at the southern coastal area of Korea from December 2011 to December 2012 (Fig. 2).

Sediment and water samples for the enumeration of cyst potential and cells development were collected bimonthly from the stations 1 and 2 near Chilcheon Island in Jinhae Bay from Feb to May 2013. the latitude and longitude were determined continuously at the study site (Table 1, Fig. 3).

2.2 Observation of physicochemical parameters

Water temperature, salinity, pH, DO were measured by using the hydrometer MS 5 (Hach Hydro lab). quantitative water samples were taken from the surface and bottom using a water sampler and phytoplankton net (Ø 20 μm nylon mesh) and concentrated into 50 mL volume and were fixed with 1% glutaraldehyde solution (1 mL).

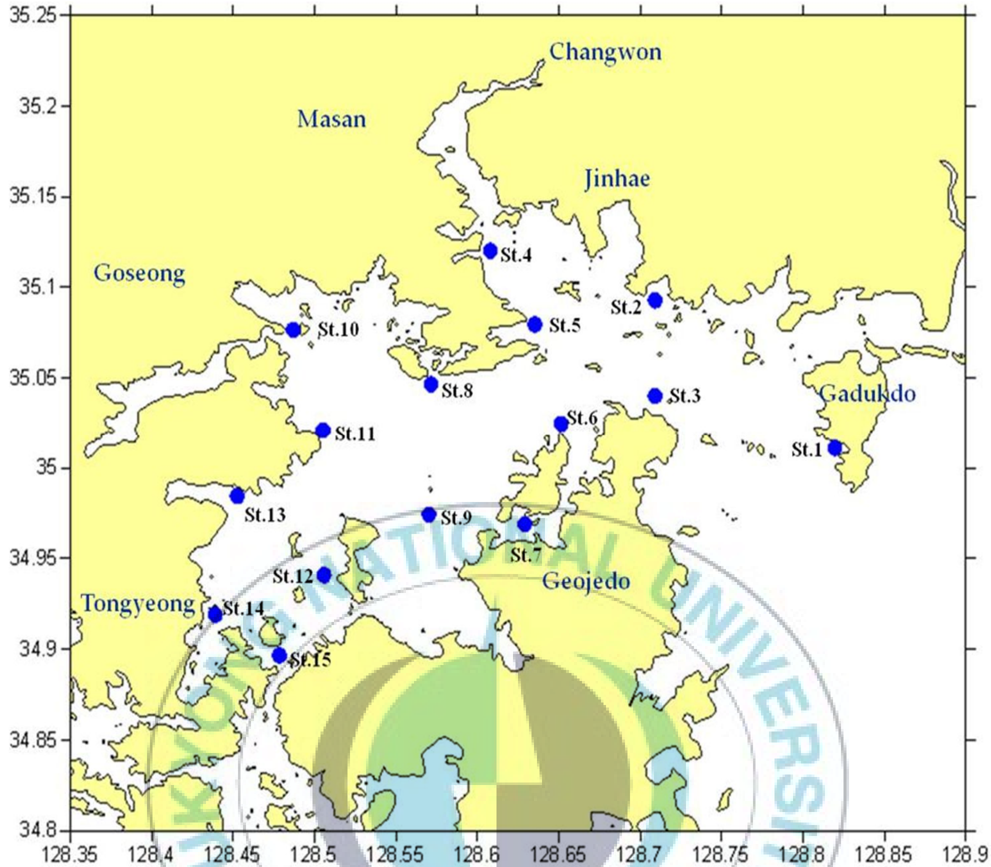

Fig. 2. Periodic sampling stations of bottom sediment by NFRDI in Jinhae Bay, Korea (modified from the National Fisheries Research & Development Institute).

Table 1. Location of sampling stations in Chilcheon Island, Jinhae Bay from Feb to May 2013

Station	Latitude	Longitude
CCSt. 1	35° 01′ 90″ N	128° 64′ 35″ E
CCSt. 2	35° 01′ 72″ N	128° 63′ 88″ E

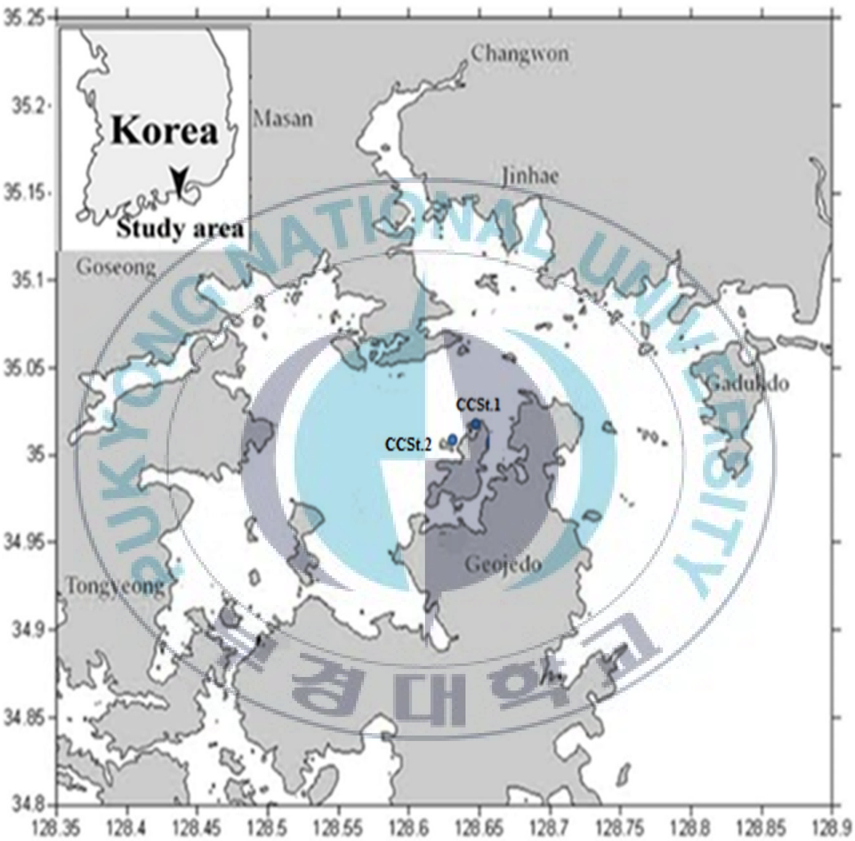

Fig. 3. Map showing the periodic (bimonthly) sampling stations near Chilcheon Island in Jinhae Bay from Feb to May 2013.

2.3. Sediment samples treatment

Sediments from NFRDI were placed into small plastic bottles and stored at 4 °C in dark place until they were used. Ten grams (wet weight) of each sediment samples were suspended in filtered sea water (FSW) and sieved through a nylon mesh to obtain the size fraction measuring between 20 μm and 125 μm, the materials remaining on the net mesh size-20 μm was washed with FSW in 15 mL centrifuge tubes until 7 mL and inject 3 mL of SPT (sodium polytungstate) solution using injector carefully beneath the sediment (Fig. 4).

The suspension ingredient was centrifuged at 1600×g for 10 min. after centrifugation, we got the organic materials including resting cysts of *Alexandrium* spp. by using micropipette, and added it into a 15 mL tube with 12 mL of filtered seawater and centrifuged at 1000×g for 2 min × 3 times (Fig. 5). the final 2 mL were taken and cyst counts were done using counting chamber under the microscope equipped at 100X magnification.

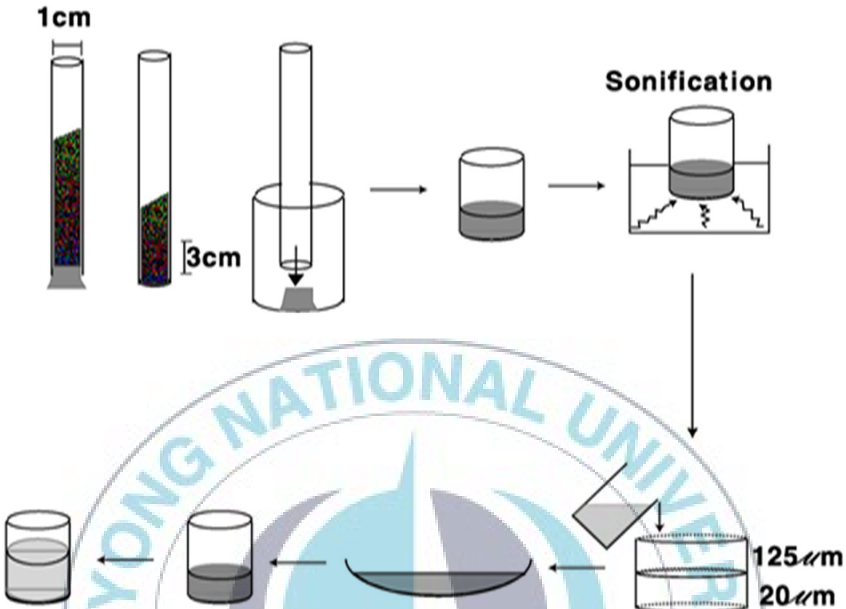

Fig. 4. Procedure for the cyst concentration from sediment samples (modified from Matzuoka and Fukuyo, 2000).

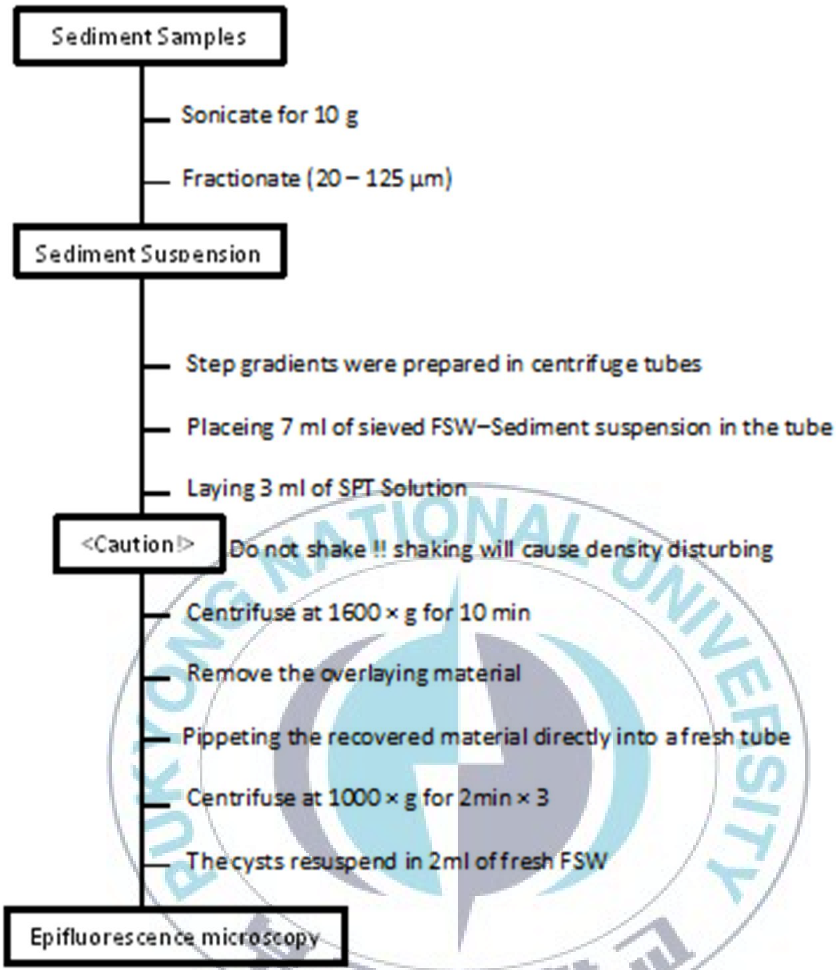

Fig. 5. Protocol of SPT (Sodium polytungstate) treatment method for the enumeration of *Alexandrium* resting cysts in natural sediments (modified from Matzuoka and Fukuyo, 2000).

2.4. Statistical analysis

Statistical analyses (means, variances, standard deviations) were carried out using the software SPSS for the variances. One-way (ANOVA) was used to test for the mean different between sampling stations and months, and multiple comparison analysis was also carried out using Tukeys HSD and Duncan for comparison error test between means of all results.

3. Results

3.1 Variation of physical factors

Water temperature (°C) averaged on the periodic sampling stations in Jinhae Bay from Dec 2011 to Dec 2012 shows a clear seasonal variation pattern with lower ranges in winter and higher in summer. subsequently spring and autumn exist as transitions between summer and winter. Spring has lower mean temperatures than autumn across year. (Table 2, Fig. 6)

Also on the physical factors such as temperature (°C), pH and DO (mg/L) observed at the stations near Chilcheon Island in Jinhae Bay from Feb to May 2013 indicated for the highest mean in the summer season and the lowest in the winter (Figs. 7-12 and Tables 3-6), while the salinity ranges high in winter and low during spring season (Figs. 12-13).

Table 2. Mean value of temperature in Jinhae Bay from Dec 2011 to Dec 2012

Location	Date	Temp (°C)
Jinhae Bay	Dec 2011	12.75
	Jan 2012	8.30
	Feb 2012	6.75
	Mar 2012	9.35
	Apr 2012	12.25
	May 2012	20.15
	Jun 2012	21.45
	Jul 2012	24.85
	Aug 2012	27.10
	Sep 2012	25.75
	Oct 2012	20.85
	Nov 2012	17.25
	Dec 2012	13.65

Data source: NFRDI home page (www.nfrda.re.kr)

Fig. 6. Monthly variation of water temperature (°C) averaged on the periodic sampling stations in Jinhae Bay from Dec 2011 to Dec 2012.

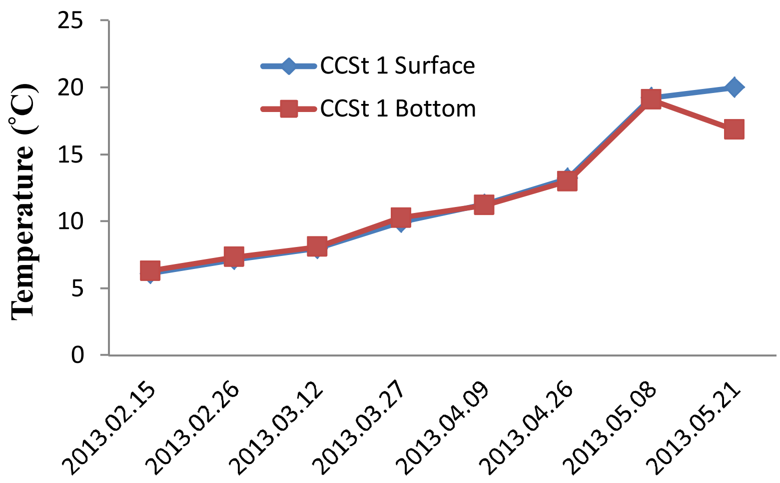

Fig. 7. Monthly variation of water temperature averaged at surface (◆) and bottom (■) of the sampling station (CCSt. 1) from Feb to May 2013.

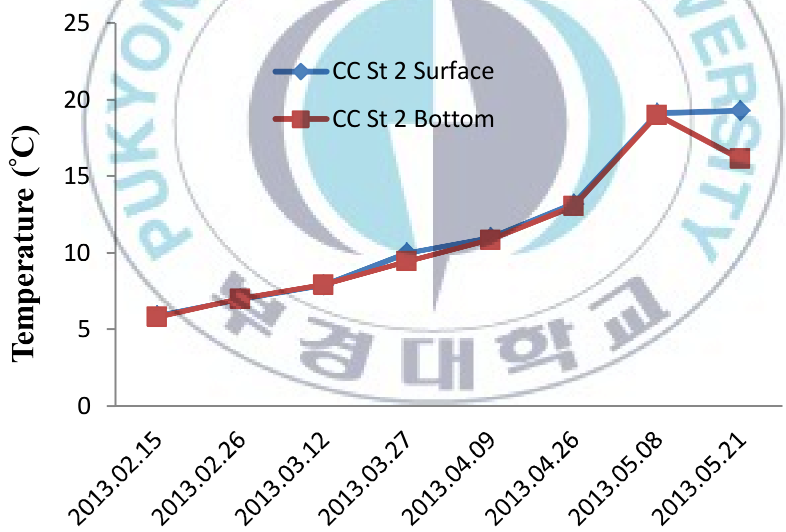

Fig. 8. Monthly variation of water temperature averaged at surface (◆) and bottom (■) of the sampling station (CCSt. 2) from Feb to May 2013.

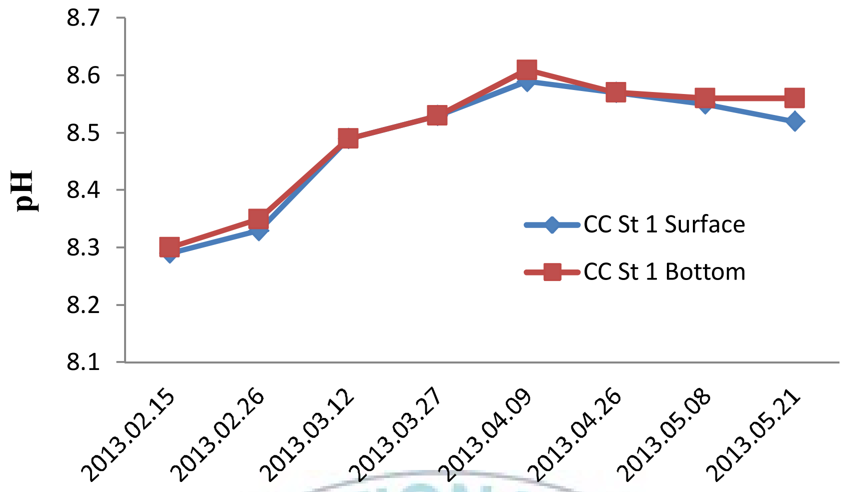

Fig. 9. Monthly variation averaged of pH at surface (◆) and bottom (■)of the sampling station (CCSt. 1) from Feb to May 2013.

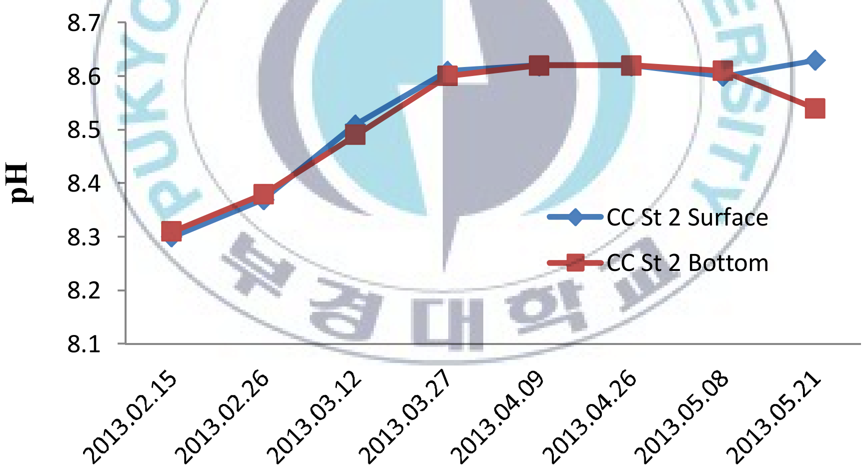

Fig. 10. Monthly variation averaged of pH at surface (◆) and bottom (■) of the sampling station (CCSt. 2) from Feb to May 2013.

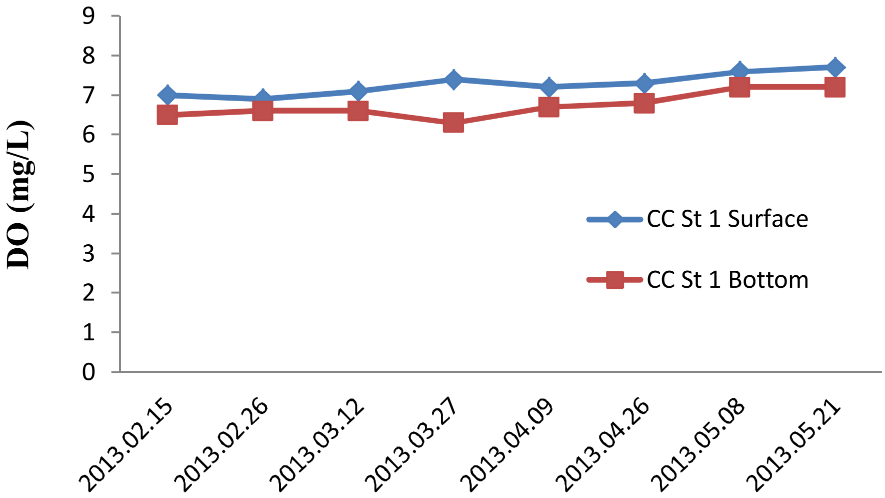

Fig. 11. Monthly variation averaged of DO at surface (◆) and bottom (■) of the sampling station (CCSt. 1) from Feb to May 2013.

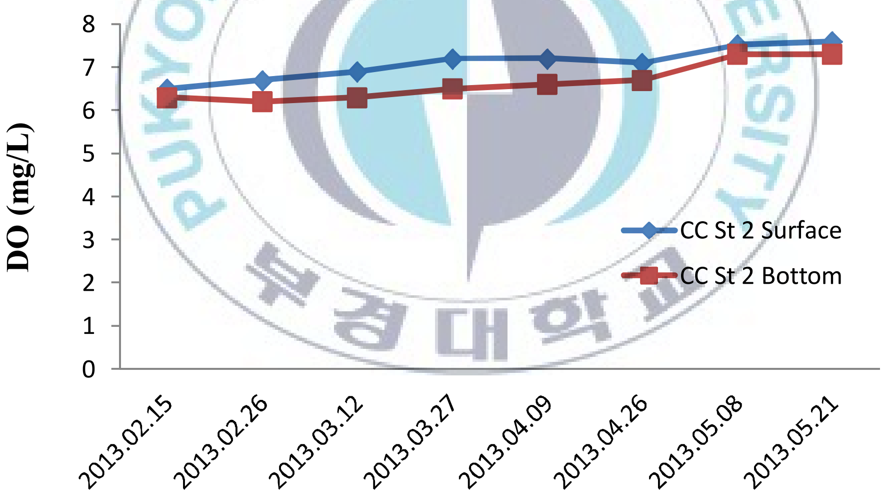

Fig. 12. Monthly variation averaged of DO at surface (◆) and bottom (■) of the sampling station (CCSt. 2) from Feb to May 2013.

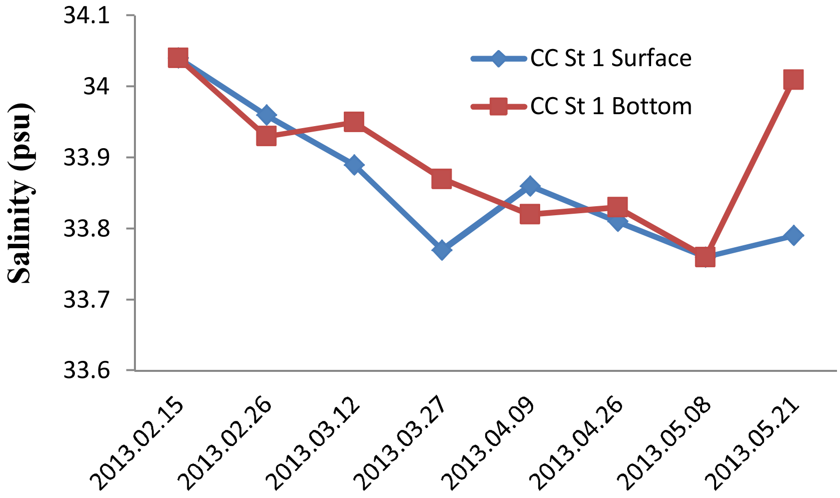

Fig. 13. Monthly variation averaged of salinity (psu) at surface (◆) and bottom (■) of the sampling station (CCSt. 1) from Feb to May 2013.

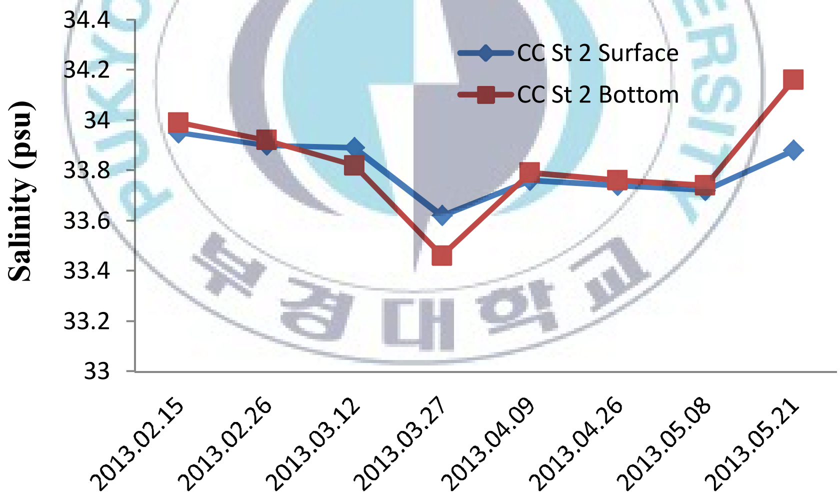

Fig. 14. Monthly variation averaged of salinity (psu) at surface (◆) and bottom (■) of the sampling station (CCSt. 2) from Feb to May 2013.

Table 3. Mean value of environmental factors at the sampling stations in Jinhae Bay at February 2013

Station	Temp (°C)	Sal (psu)	pH	DO (mg/L)
CCSt. 1 Surface	6.64	34.00	8.31	6.95
CCSt. 1 Bottom	6.84	33.99	8.35	6.55
CCSt. 2 Surface	6.44	33.93	8.34	6.60
CCSt. 2 Bottom	6.44	33.96	8.35	6.25

Table 4. Mean value of environmental factors at the sampling stations in Jinhae Bay at March 2013

Station	Temp (°C)	Sal (psu)	pH	DO (mg/L)
CCSt. 1 Surface	8.98	33.78	8.51	7.25
CCSt. 1 Bottom	9.2	33.91	8.51	6.25
CCSt. 2 Surface	8.95	3376	8.56	7.05
CCSt. 2 Bottom	8.69	33.64	8.55	6.4

Table 5. Mean value of environmental factors at the sampling stations in Jinhae Bay at April 2013

Station	Temp (°C)	Sal (psu)	pH	DO (mg/L)
CCSt. 1 Surface	12.26	33.84	8.58	7.25
CCSt. 1 Bottom	12.12	33.83	8.59	6.75
CCSt. 2 Surface	12.09	33.75	8.62	7.15
CCSt. 2 Bottom	11.95	33.78	8.62	6.65

Table. 6. Mean value of environmental factors at the sampling stations in Jinhae Bay at May 2013

Station	Temp (°C)	Sal (psu)	pH	DO (mg/L)
CCSt. 1 Surface	19.06	33.51	8.51	7.65
CCSt. 1 Bottom	18	33.92	8.56	7.2
CCSt. 2 Surface	19.19	33.8	8.62	7.56
CCSt. 2 Bottom	18	33.95	8.58	7.3

3.2. Spatio-temporal distribution of *Alexandrium* cysts

Alexandrium spp. cysts were found in 182 sediment samples collected by National Fisheries Research and Development Institute (NFRDI) at 14 stations in Jinhae Bay from Dec 2011 to Dec 2012, but the abundance was highly variable in a concentration range of 14-250 cysts/cm^3 by showing spatio-temporal distribution patterns (Tables 7-8, Figs. 15-28).

The abundance of *Alexandrium* cysts varied horizontally among the sampling stations in Jinhae Bay where the highest concentration was found in St.11 (141.5±51.9 cysts/cm^3) and relatively higher distribution pattern showed around Jindong Bay stations of St. 10, St. 5 and St. 8 (Fig. 29, Table 9).

According to Duncan and Turkeys multiple comparison, the cyst abundance appeared to be highly variable among months, but it showed conspicuous variation patterns at all stations, whereas the majority of the cysts counted were observed high from December to February in winter season, and June at the end of spring time. The highest mean abundance (76.6±56.7 cells/cm^3) in February was followed by a conspicuous decrease till April to May, and a sudden increase at all stations appeared in June. (Table. 10, Figs. 15-28).

The statistical analysis results in this study gave a P-value lower 0.05 among the difference seasons. Winter season had been highest cysts (Mean±SD; 67.52±43.952), but that there was significant difference among variable seasons (winter, spring, summer and autumn) (Table 11) in the samples that collected from Dec 2011 to Dec 2012 by NFRDI in Jinhae Bay.

Cysts were counted in July is very low density in NFRDI sediment samples in Jinhae Bay at all 14 stations (Table 7).

Their abundance showed the highest value comparable to the previous and later months of May and July which showed nearly the lowest values in the year. this patterns in monthly cyst abundance is a simmilar result to the previous distribution and germination experiments that there were high densities in June and low July (Shin, 2002).

Table 7. The distribution of *Alexandrium* spp. cysts/cm^3 in sediment samples collected by NFRDI in Jinhae Bay from December 2011 to May 2012

Stations	Month/Year						Total
	Dec 2011	Jan 2012	Feb 2012	Mar 2012	Apr 2012	May 2012	
St.2	43	53	60	39	25	26	246
St.3	35	43	54	35	30	35	232
St.4	20	24	35	23	20	22	144
St.5	95	100	113	55	47	58	468
St.6	34	55	57	53	36	38	273
St.7	25	33	40	26	29	28	181
St.8	66	66	70	56	48	57	363
St.9	65	63	77	49	39	45	338
St.10	105	96	124	90	50	69	534
St.11	150	170	250	120	130	136	956
St.12	45	44	50	35	28	32	234
St.13	32	32	35	30	27	25	181
St.14	65	63	69	39	35	40	311
St.15	27	29	39	24	18	22	159
Total	807	871	1073	674	562	633	4620

Table 8. The distribution of *Alexandrium* spp. cysts/cm^3 in sediment samples collected by NFRDI in Jinhae Bay from June 2012 to December 2012

Stations	Month/Year							Total
	Jun 2012	Jul 2012	Aug 2012	Sep 2012	Oct 2012	Nov 2012	Dec 2012	
St.2	50	15	18	18	20	30	52	203
St.3	49	20	28	25	25	38	47	232
St.4	36	18	27	27	29	34	42	213
St.5	70	45	50	47	47	62	110	431
St.6	52	37	40	37	45	47	54	312
St.7	43	27	29	23	40	55	58	275
St.8	75	46	53	50	55	59	72	410
St.9	73	14	16	26	33	60	70	292
St.10	120	62	75	64	70	90	110	591
St.11	200	73	82	87	115	126	200	883
St.12	50	29	39	38	45	47	55	303
St.13	33	25	27	25	30	38	40	218
St.14	67	32	39	35	54	50	75	352
St.15	42	19	23	25	30	35	45	219
Total	960	462	546	527	638	771	1030	4934

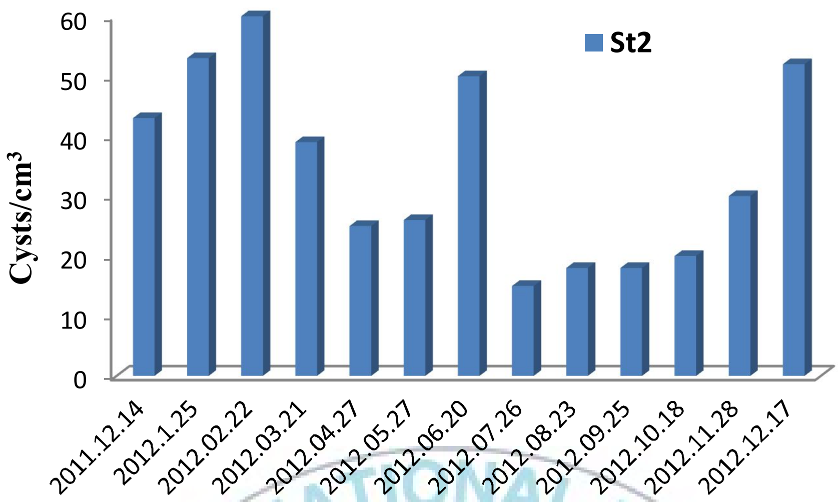

Fig. 15. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 2 from Dec 2011 to Dec 2012.

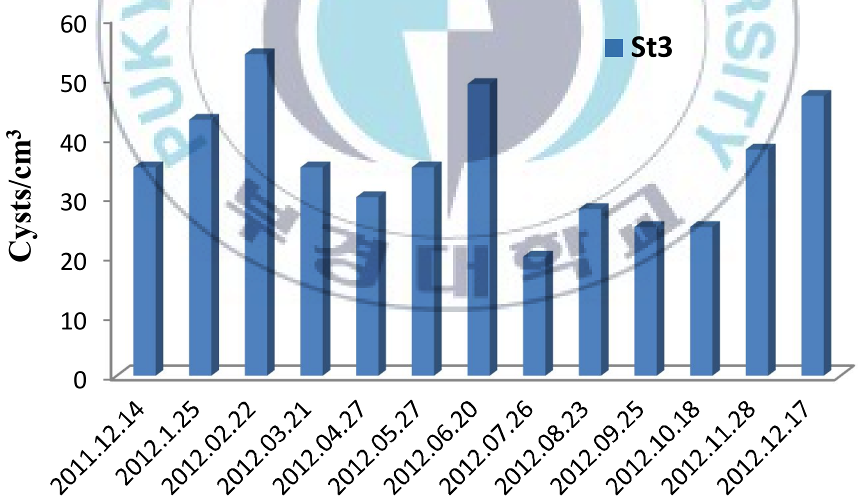

Fig. 16. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 3 from Dec 2011 to Dec 2012.

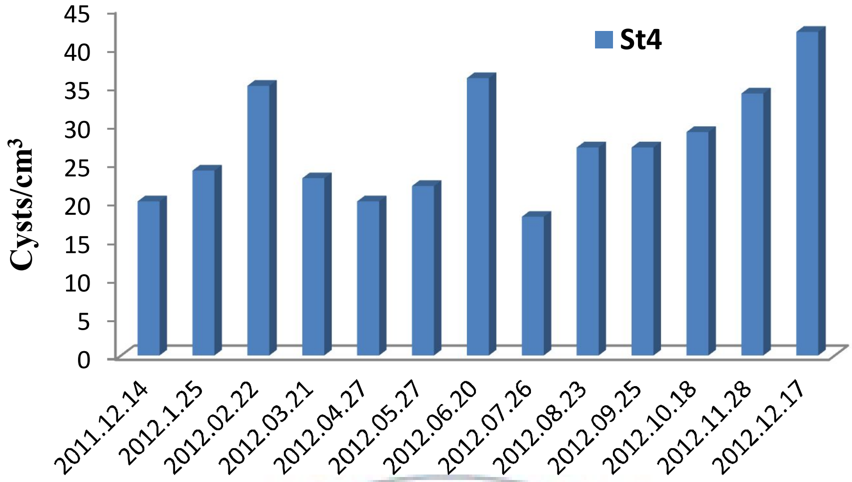

Fig. 17. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 4 from Dec 2011 to Dec 2012.

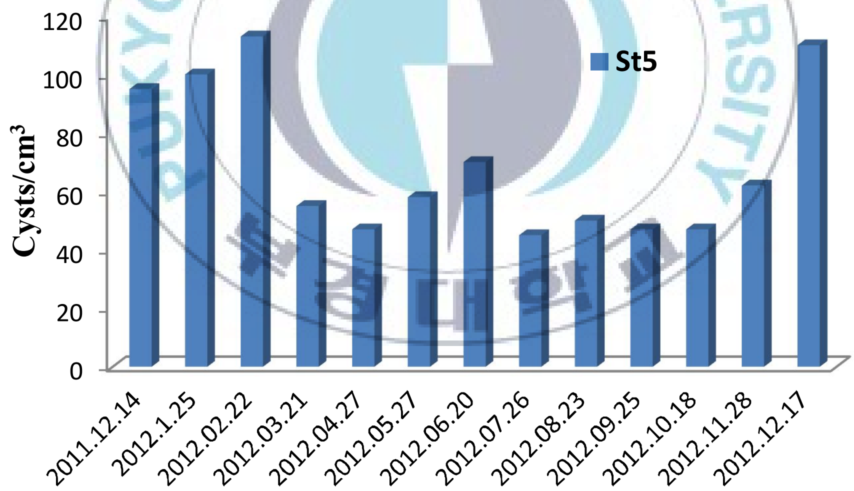

Fig. 18. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 5 from Dec 2011 to Dec 2012.

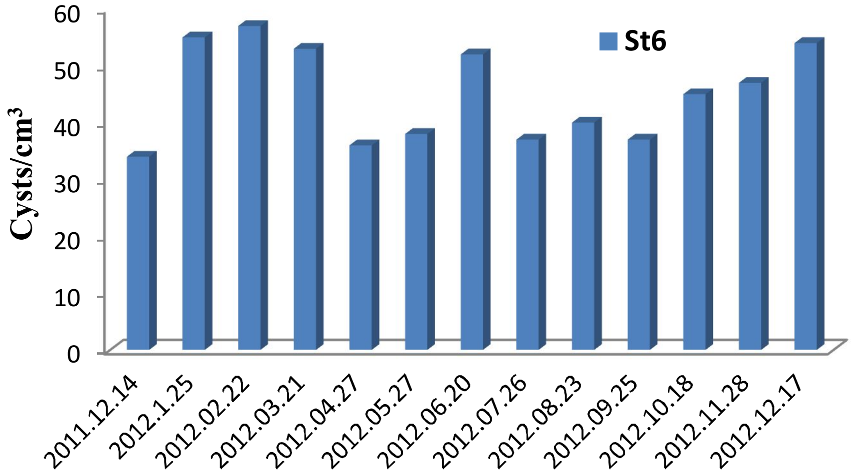

Fig. 19. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 6 from Dec 2011 to Dec 2012.

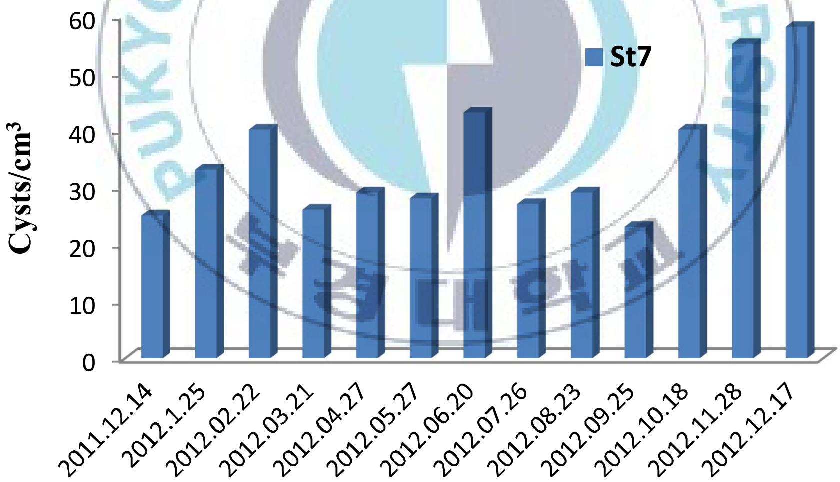

Fig. 20. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 7 from Dec 2011 to Dec 2012.

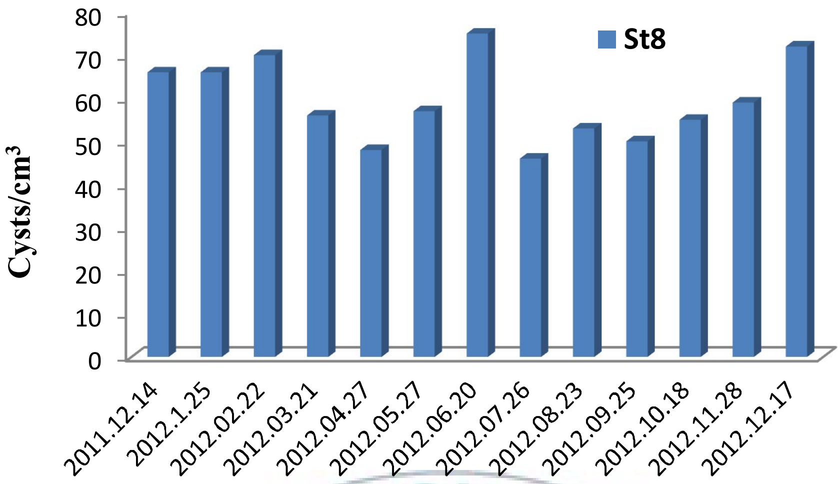

Fig. 21. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 8 from Dec 2011 to Dec 2012.

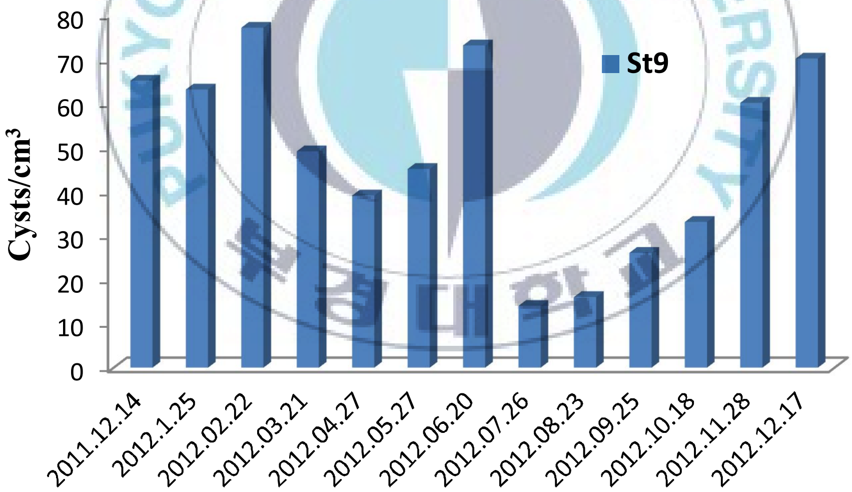

Fig. 22. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 9 from Dec 2011 to Dec 2012.

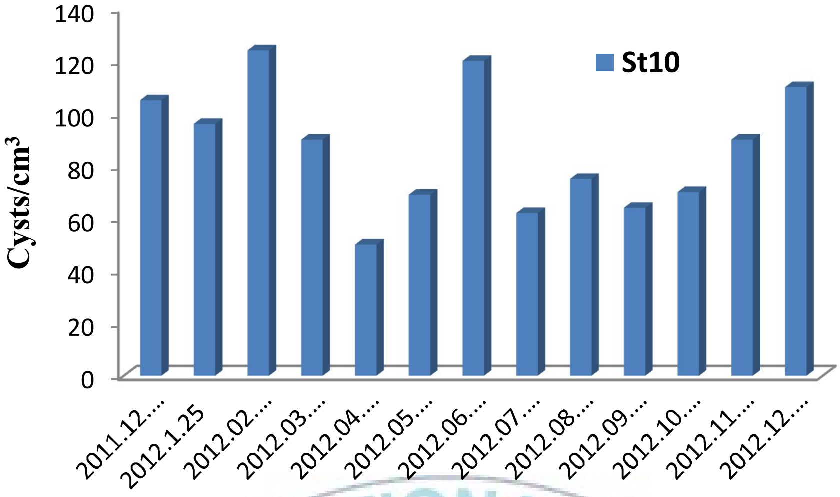

Fig. 23. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 10 from Dec 2011 to Dec 2012.

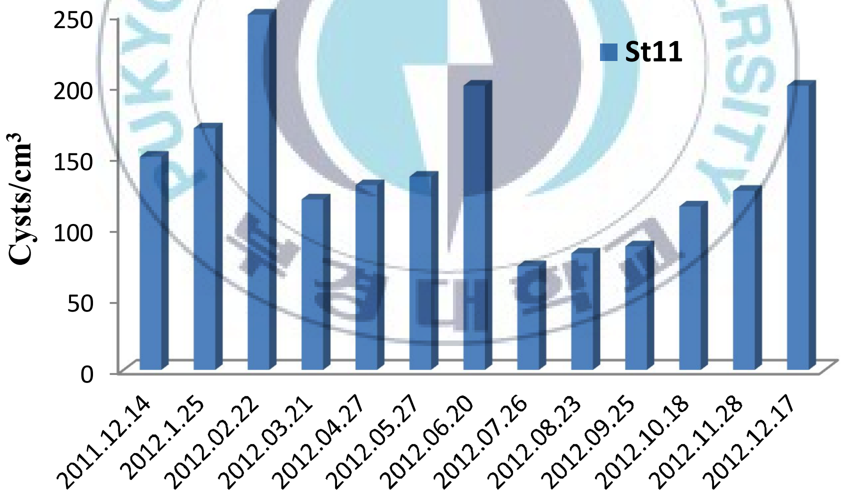

Fig. 24. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 11 from Dec 2011 to Dec 2012.

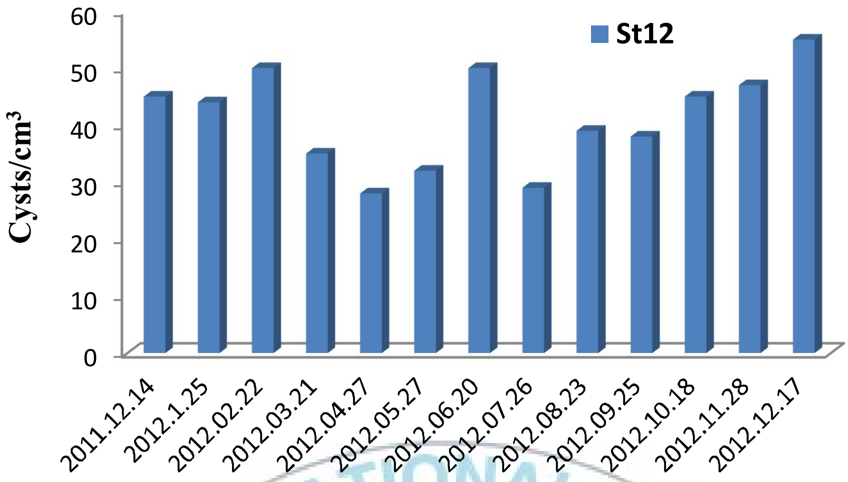

Fig. 25. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 12 from Dec 2011 to Dec 2012.

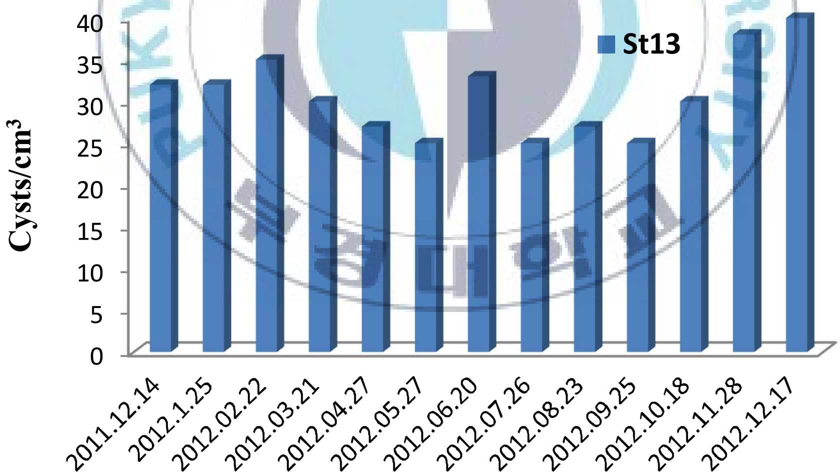

Fig. 26. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 13 from Dec 2011 to Dec 2012.

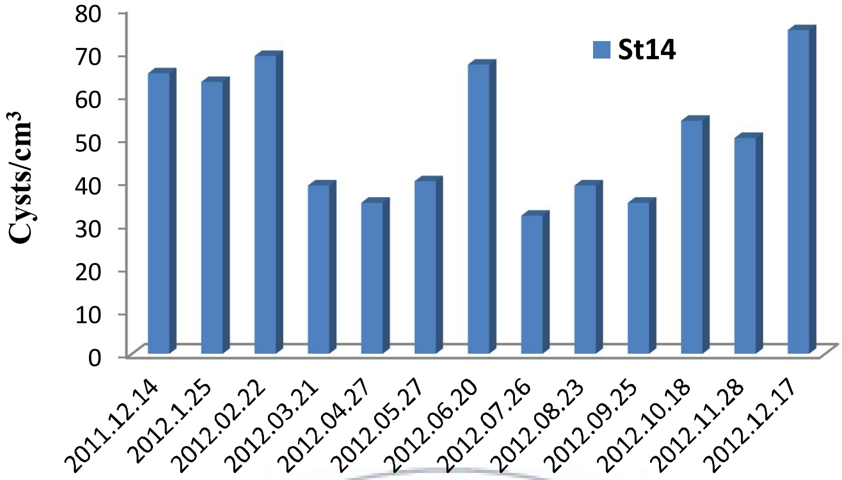

Fig. 27. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 14 from Dec 2011 to Dec 2012.

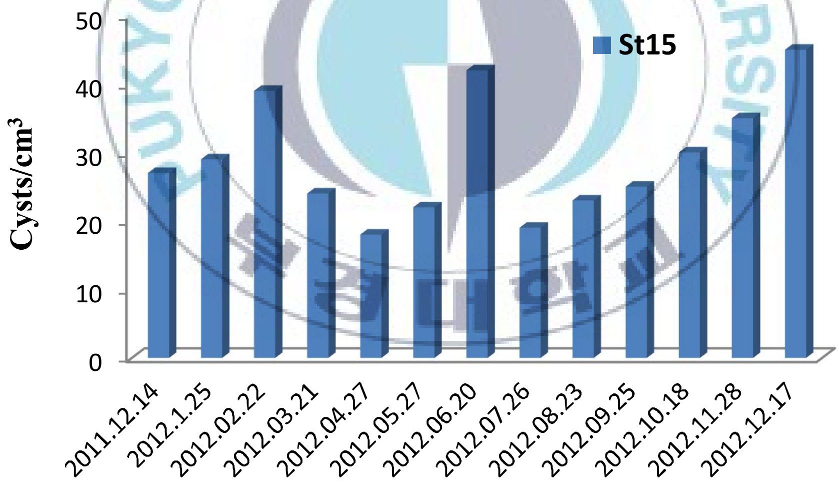

Fig. 28. Monthly variation of the abundance of *Alexandrium* spp. cysts in NFRDI sediment samples at St. 15 from Dec 2011 to Dec 2012.

Table 9. Results of SPSS test for the abundance of *Alexandrium* spp. cysts /cm^3 among the stations in sediment samples by NFRDI from Dec 2011 to Dec 2012

Dependent variable : cyst abundance/cm^3 at stations							
Stations	N	Mean	SD	Stations	N	Mean	SD
St.2	13	34.5	15.7	St.9	13	48.5	21.6
St.3	13	35.7	10.3	St.10	13	86.5	23.6
St.4	13	27.5	7.4	St.11	13	141.5	51.9
St.5	13	69.2	23.6	St.12	13	41.3	8.5
St.6	13	45	8.4	St.13	13	30.7	4.9
St.7	13	35.1	11.4	St.14	13	51	15.2
St.8	13	59.5	9.5	St.15	13	29.1	8.7
Total					182	52.5	335.2

Sum of Squares	df	Mean Square	F	P
158060.264	13	12158.482	31.057	.000
4435.802	1	4435.802	11.331	.001
153624.462	12	12802.038	32.701	.000
65769.231	168	391.484		
223829.495	181			

R^2 =0.86 P = 0.0001 N= number of samples.

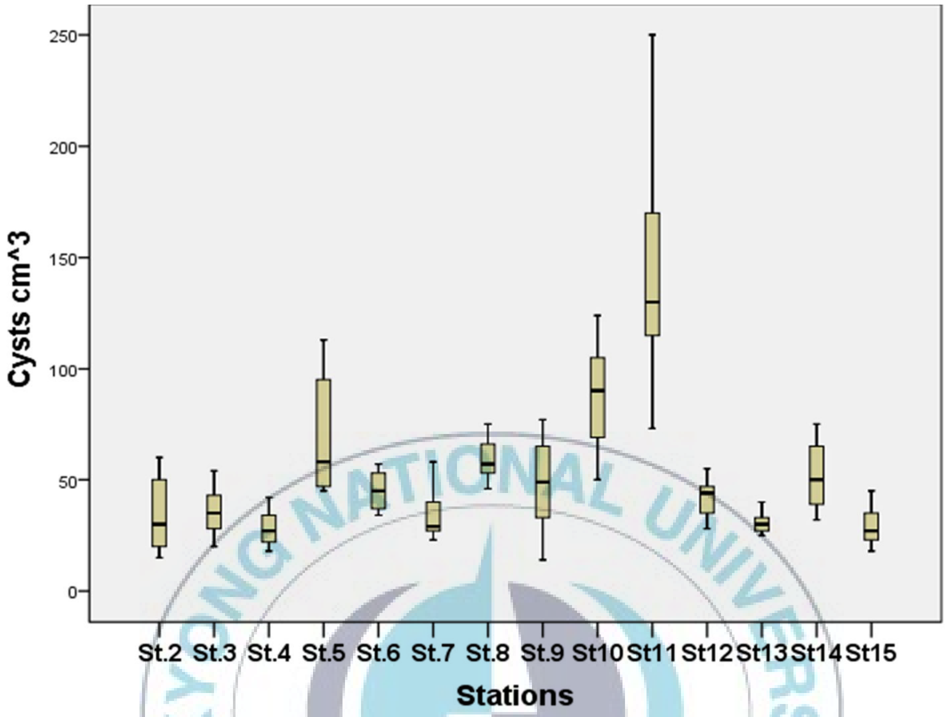

Fig. 29. The abundance of *Alexandrium* spp. cysts/cm^3 in sediment samples collected from the periodic monitoring stations by NFRDI in Jinhae Bay from Dec 2011 to Dec 2012. One-way ANOVA test showed significant different ($p<0.05$) among the mean value of all stations.

Table 10. Results of SPSS test for a comparison of *Alexandrium* cyst abundance in the monthly mean value of 14 stations in Jinhae Bay. Sediment samples were collected by NFRDI from Dec 2011 to Dec 2012

Dependent variable : Cysts abundance/cm^3 in Months

Month	N	Mean	SD	Month	N	Mean	SD
Jan	14	62.2	38.6	Jul	14	33	17.9
Feb	14	76.6	56.7	Aug	14	39	19.9
Mar	14	48.1	27.2	Sep	14	37.6	19.
Apr	14	40.1	27.7	Oct	14	45.6	24.2
May	14	45.2	29.9	Nov	14	55	25.7
Jun	14	68.6	43.9	Dec	14	73.6	42.7
Corrected Total				168	52	35.08	2.7

	Sum of Squares	df	Mean Square	F	P
	34158.494	11	3105.318	2.826	.002
	579.923	1	579.923	.528	.469
	33578.571	10	3357.857	3.056	.001
	171397.786	156	1098.704		
Total	205556.280		167		

R^2 =0.86 P=0.0001 N= number of samples.

Table 11. Results of SPSS test for the abundance of *Alexandrium* spp. cysts/cm^3 among the seasons in sediment samples by NFRDI from Dec 2011 to Dec 2012

Dependent variable : Cysts abundance/cm^3 among the seasons								
Season	N	df	Mean	SD	Sum of Squares	Mean Square	F value	P
winter	56	3	67.52	43.952	18378.250	6126.083	5.308	.002
spring	42	3	44.50	27.794	9069.484	9069.484	7.858	.006
summer	42	3	46.86	32.956	10917.880	10917.880	9.459	.002
fall	42	3	46.10	23.684	7460.371	3730.185	3.232	.042
Corrected Total	182		52.49	35.166	46179.11	29843.63		

N= number of samples.

3.3. A Relationship between motile cells appearance and cysts abundance during vernal warming period

Cysts and vegetative cells were also present at two stations of CCSt. 1 and CCSt. 2 near ChilCheon Island in Jinhae Bay from February to May 2013.

By the results of bimonthly monitoring of motile cells occurrence and cysts abundance at two stations near Chilcheon Island, there was a close relationship between the occurrence of vegetative cells and the abundance of cysts. Vegetative cells of *Alexandrium* spp. were found both in surface and bottom sea water samples and at two stations (CCSt. 1 and CCSt. 2) during the surveyed period, but no significant difference between the two stations (Tables 14-15) (Figs. 30-31). Their densities were very low in February (3.8±1.7 cells/mL) and reached to maximum concentration in late April (13.6±2.8 cells/mL) (Table 12, Figs. 14-15)

Although the cysts were present at stations CCSt.1 and CCSt.2 in sediment sampled that was bimonthly collected from Chilcheon Island in Jinhae Bay during February, March, April and May 2013. T.Test analysis showed no significant difference ($P>0.05$) in the cysts abundance between two stations during the survey, but higher concentrations were generally found in the station CCSt. 2 (Mean±SD, 33.9±8.4) than CCSt.1 (Mean±SD, 29.9±7.7) ((Table 16, Figs. 30-31).

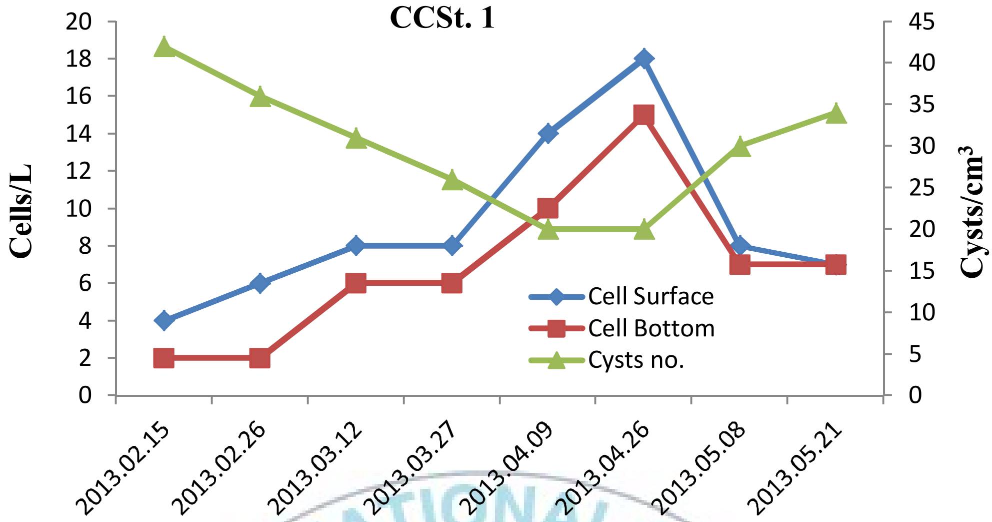

Fig. 30. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at station CCSt. 1 in Jinhae Bay from Feb to May 2013.

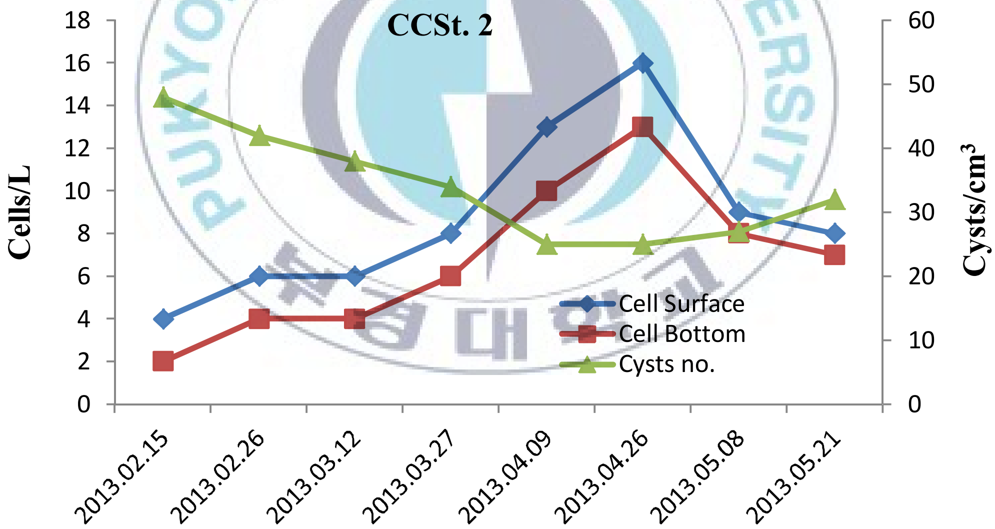

Fig. 31. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at station CCSt. 2 in Jinhae Bay from Feb to May 2013.

Table 12. Results of SPSS of one-way (ANOVA) test for the monthly abundance of *Alexandrium* spp. cells/L at two stations of CCSt. 1 and CCSt. 2 in Jinhae Bay from Feb to May 2013

	Dependent variable : Monthly abundance of cells/L			
Month	N*	Mean	SD	P-value
Feb	8	3.8	1.7	
Mar	8	6.5	1.4	.043
Apr	8	13.6	2.8	
May	8	7.6	.74	

* N= number of samples.

SPSS analysis on the variance showed a significant difference (P <0.05) in the cells abundance at two stations of CCSt. 1 and CCSt. 2 from Feb to May 2013.

Table 13. Results of SPSS of one-way (ANOVA) test for the monthly abundance of *Alexandrium* spp. cysts/cm^3 at two stations of CCSt. 1 and CCSt. 2 in Jinhae Bay from Feb to May 2013

	Dependent variable : Monthly abundance of cysts/cm^3			
Month	N	Mean	SD	P-value
Feb	8	42.0	4.9	
Mar	8	32.3	5.1	0.802
Apr	8	22.5	2.9	
May	8	30.8	3	

SPSS analysis on the variance showed a significant difference (P <0.05) in the cysts abundance of sediment samples at two stations (CCSt. 1 and CCSt. 2) from Feb to May 2013.

Table 14. T.Test for a comparison of the abundance of *Alexandrium* spp. cells in surface water between two stations (CCSt. 1 and CCSt. 2) in Jinhae Bay from Feb to May 2013

	Dependent variable : The abundance of surface cells/L			
Station	N	Mean	SD	P-value
CCSt. 1	8	9.13	4.5	.704
CCSt. 2	8	8.75	4	

T.Test analysis showed no significant difference (P>0.05) in the abundance of surface cells between two stations during the survey periods.

Table 15. T.Test for a comparison of the abundance of *Alexandrium* spp. cells in bottom water between two stations (CCSt. 1 and CCSt. 2) in Jinhae Bay from Feb to May 2013

	Dependent variable : The abundance of bottom cells/L			
Station	N	Mean	SD	P-value
CCSt.1	8	6.9	4.2	.922
CCSt.2	8	6.8	3.6	

T.Test analysis showed no significant difference (P>0.05) in the abundance of surface cells between two stations during the survey periods.

Table 16. T.Test for a comparison of the abundance of *Alexandrium* spp. cysts/cm^3 in the sediment between two stations (CCSt. 1 and CCSt. 2) in Jinhae Bay from Feb to May 2013

	Dependent variable : the abundance of cysts/cm^3			
Station	N	Mean	SD	P-value
CCSt.1	8	29.9	7.7	.8
CCSt.2	8	33.9	8.4	

T.Test analysis showed no significant difference ($P>0.05$) in the cysts abundance between two stations during the survey periods.

4. Discussion

Among the causative algal species, some of them belonging to a group called dinoflagellates and especially to the genus *Alexandrium*, can produce potent paralyzing toxins which effects can be deathful for humans. these toxins are accumulated in filter-feeding shellfish that feed upon these toxic algae; these toxins arrive to human consumer of these contaminated shellfish. Their impact is very important in the coastal waters of Korea, with dangerous level of accumulated toxins in shellfish exceeded every year in many areas. Actually two PSP accidents that resulted in human deaths were reported from Korea in 1986 and 1996 (Chang et al., 1987; Lee et al., 1997). Chang et al. (1988) first reported a correlation between the occurrence of *A. tamarense* (as *Protogonyaulax tamarensis*) and shellfish intoxication, and Han et al. (1992) demonstrated that the causative microorganisms are *A. tamarense*. in addition, *G. catenatum* was also known to produce PSP toxins in Korea (Kim and Shin, 1997; Park et al., 2004).

Alexandrium occurrence and the resting cyst abundance were surveyed and monthly surveys were continued on *Alexandrium* in one station of Jinhae Bay since 1996 (Kim and Shin, 1997). swimming cells of *A. tamarense* appear in January and disappear in May or June. mostly there appeared the most standing crops in April, when water temperature begins to rise and when red tide was formed by this species in 1997. Concentration of vegetative cells can be determined by proliferation by environmental factors such as water temperature, but the inoculum of vegetative cells into the water was initiated by germination of resting cysts when water temperature was the lowest in the year and lasted about two months. concentration of vegetative cells showed conspicuously high after resting cysts decreased sharply in the sediments. germination of resting cysts had a lot of influence on regional population and quantitative variation, and the germination rate showed the highest periodicity in January and February of the year. these results reflected that the germination of resting cysts was controlled by endogenous biological clock. the same results were reported in Maine Bay, America and St. Lawrence estuary, Canada (Anderson and Keafer 1987; Perez et al. 1998), and germination was induced by endogenous annual clock where there is no or little influence by changes of outer environments such as water temperature, day length.

In this study, when the bloom potentials of *Alexandrium* spp. were enumerated among the 14 stations in Jinhae Bay from December 2011 to December 2012, higher abundance of cysts concentration was observed in stations St.11 (141.5±51.9 cysts/cm^3) and St. 10, St. 5 and St. 8 around Jindong Bay (Fig. 29, Table 9) where is a relatively deeper central area than the other places in Jinhae. this result is consistent with the result of Matrai et al. (2005) that *Alexandrium* spp. cysts more abundance in deep waters. the distribution of dinoflagellate cysts is governed not only by environmental factors such as the particle size of sediment, sedimentation rate, and hydrographic and geographical features, but also by biological factors (Dale 1976, White and Lewis 1982, Turgeon et al. 1990). cysts behave as fine-grained sedimentary particles, and move and concentrate along hydrodynamic systems by the winnowing effects of neritic suspended particles containing various micro flora and micro fauna. also Jinhae Bay has many different water depth ranging from 5-20 meters and strong water currents with speed 100 cm/s and the general hydrographic features are characterized by the northward inflow of the Tsushima current and the subsequent westward flows Dangdong to Tongyeong (Lee, 1998). thus, surface sediment distributions are under the direct influence of the centrifugal force of this current movement, and the largest fine-grained muddy deposits are located around Jindong areas in central Jinhae Bay.

Very low concentrations of vegetative cells were found in February at two stations in Chilcheon Island in Jinhae Bay (Figs. 30-31). the endogenous clock causes germination to begin at February, so the cells we observed might have recently germinated or might have continued through the winter months. since 1994 Kim's laboratory have been focused on Jinhae Bay to find out the reason for paralytic shellfish poisoning and proved the relationship between the occurrence of *Alexandrium* as causative organisms and shellfish toxification (Kim 1994; Kim 1995; Kim and Lee 1996). *Alexandrium* was usually found at the low temperature and disappeared around May in Jinhae Bay station, but in 1996 it continued to occur even in June, causing long-term toxification and also occurred massively in September, as was not the case with before. moreover, a high concentration of *Alexandrium tamarense* blooms occurred in April, 1997, that is 7.2×10^6 cells/L, and red tide continued for 20 days in Chindong Bay. the period from December 1998 to April 1999 showed a constant occurrence but a relatively low occurrence in the surface and bottom layer, that is, under 4,800 cells/L (April, in the surface). in the year, the winter occurrence began in December and continued until April, next year, and the summer occurrence began in August and became maximum concentration in

September and faded away in October and the winter occurrence began again in December, when water temperature was getting low. *Alexandrium,* which occurred during the survey period of March 1998 through April 1999, showed a similar occurrence to that of Kim and Shin (1997), but this study found no *Alexandrium* genus in the two survey stations in June and July after spring. however, *Alexandrium* was observed to appear in June 1999 in Sujeongri, Jinhae Bay, unlike in 1998. therefore, it can be said that there exist a trifle different periods in different years. Toxic species appears in winter through spring and non-toxic *Alexandrium* species in fall.

Likewise, development of *Alexandrium* spp. in Jinhae Bay showed the yearly and monthly differences in initiation, abundance and lasting time. light is very limited in January and turbulence is high, although germination is possible in February, but growth conditions are poor for *Alexandrium* spp. vegetative cells and losses are likely high (Kirn et al., 2005). however, as shown in Tables 3-6 and Figs. 7-8, 13-14, the salinity and temperature in Jinhae Bay is very high in February, but low average of temperature and the subsequent elevation at the end of springtime, these higher water temperature and low in spring salinities have been lead to high concentration for *Alexandrium* spp. cells/L in April, it is also well matching with the observation in Jinhae Bay by Shin et al. (2010).

PSP toxification occurring massively in spring, especially, has a close relationship with the occurrence period of these toxic species, and swimming cells appear several months before April, when toxin concentration of shellfish is maximum. therefore, the case should be taken into full consideration that long-term occurrence leads to accumulation of toxin in shellfish even during the period of low water temperature.

5. Conclusion

Alexandrium spp. vegetative cells had been observed in winter months but blooms recurred every spring or summer. the studies revealed that the temperature and light seemed most important for *Alexandrium* spp. cyst germination.

The physical and environmental factors: oxygen, light, and warmer temperatures have been shown significantly enhance germination rates in many research studies (Anderson et al., 2005; Anderson and Wall., 1978)

The results show high concentration of vegetative cells in summer for *Alexandrium* spp. because the seasonal upwelling exist in the winter and the end of spring, mixing the water from depth to surface, when light levels are low and strong winds in the winter drive down and sets up again in the the abundant and blooms of *Alexandrium* spp. vegetative cells occurs in surface water in late summer.

Extensive studies on *Alexandrium* spp. ecology have been modicum in Jinhae Bay. even though the shellfish and fish farms in the Bay is much biggest than the other places in of embayment, for this reason, the National Fisheries Research Development Institute (NFRDI), maintains a sampling program for the detection of toxic dinoflagellate as well as monitoring the abundance of *Alexandrium* spp. to limit the extend of physical and ecological parameters that effect in germination and bloom rate of dinoflagellate exist and occurred in Jinhae Bay and coastal areas in Korea.

The results show that dinoflagellate with the *Alexandrium* spp. cysts assemblages in Jinhae Bay consider with environmental changes, suggesting that *Alexandrium* spp. cysts very important indicators for HABs in Korean coastal areas. also the data suggest that bloom potential of *Alexandrium* spp. the primary cause of the increase in dinoflagellate cysts concentrations in Jinhae Bay and impact the aquaculture and fish farm, because this increase affected by other environmental and physicochemical factors such as water temperature and salinity.

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