Thesis for the Degree of Master of Fisheries Science

Spatial Distribution of Resting Cysts and Vegetative Cells of PSP Toxigenic *Alexandrium* spp. in Jinhae Bay, Korea

by

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KOICA-PKNU International Graduate Program of Fisheries Science

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한국 진해만에서 마비성패독 원인생물 알렉산드리움의 발생과 휴면포자 분포

Advisor: Prof. Chang-Hoon Kim

by

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A dissertation

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February 27, 2015

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Abstract

Outbreaks of paralytic shellfish poisoning (PSP) and red tide caused by *Alexandrium* spp. have frequently been reported around Jinhae Bay in the southeastern coastal areas of Korea.

The present paper deals with the seasonal occurrence of motile cells and monthly alternation of the resting cysts of *Alexandrium* spp. and the phytoplankton population in Jinhae Bay from January to May 2014. The standing crop of phytoplankton population varied significantly with month; it ranged from 6,576 to 79,865 cells/mL and had the reach the maximum crop in January 2014.

Alexandrium motile cells in Jinhae Bay that had been known to produce PSP toxin appeared mostly in January when the temperature in low, and reached to high densities from March to May 2014. *Alexandrium* spp. resting cysts were distributed at seven stations in Jinhae Bay, but it showed a significant variation among stations, ranging from 17 to 117 cysts/cm^3 in the sediment samples.

Vegetative cells frequent in the cold season could result from cysts germination which had controlled mainly by environmental factors and biological endogenous clock. Considering PSP outbreaks with the same season as cysts germinated, it is suggested the monitoring for the prediction on prevailing regions should be undertaken based on the life cycle strategies of the causative organisms.

1. Introduction

Resting cysts play the main role in dispersal of dinoflagellates, and they play the initiation and termination of blooms, also they can survive under unsuitable conditions (Wall 1971; Anderson 1984; Steidinger 1993). Thus the researches on the distribution and abundance of cysts in sediments samples are important to understand ecology and bloom dynamics of red tide dinoflagellates, including toxic species (Anderson and Wall 1978; White and Lewis 1982).

In Korea, Paralytic Shellfish Poison (PSP) outset has been reported for the first time in 1986. Therefore, the Korean government pushed the researchers to determine the causative organisms of PSP along the southeastern coast of Korean peninsula.

Jinhae Bay is located in the southeastern coast of Korean peninsula. It is a semi-enclosed with narrow channel to the open sea. Water depth ranges from 5 m to 20 m. Jinhae Bay contains many small neighboring bays such as Jindong Bay, Heangam Bay and Gohyum Bay. Jinhae Bay has long been used for aquaculture of finfish and oysters since 1970's.

Jinhae Bay has suffered by harmful algal bloom (HABs) which has been caused by *Alexandrium* species, and its range has been wildly expanded since 1979's.

To prevent PSP the oyster and mussels are harvested from March to April on the basis of PSP toxin monitoring results (Han et al., 1993; Kim and Shin, 1997).

Harmful algae and toxic species usually appeared in spring period. Development new techniques are important to monitor of harmful algae based on its life cycle it will be helpful to warning toxification of shellfish. The life cycle of *Alexandrium* spp. is a sequence between asexual and sexual reproduction (Fig. 1). By simple deviations (binary deviation) motile cells are reproduced, as blooms developed by vegetative cells. At this point an asexual phase is completed and sexual phase induce. The sexual process begins when the gametes were formed, while those gametes fuse to form swimming zygotes (planozygotes) later change to be recumbent, resting cysts (hypnozygotes). Advantageous information about *Alexandrium* cysts morphology has well explained (Bolch et al., 1991). Yet, the cyst will turn to hypnozygotes formed through sexuality. When vegetative cells are under unfavorable conditions such as sudden change of salinity or temperature, most species produce "temporary cyst" which is another resting cyst stage. When the conditions turn to favorable, the temporary cysts quickly re-germinate as vegetative and motile cells. Thus, the temporary resting state allows the cells to resist environmental changes in the short term (Anderson, 1998).

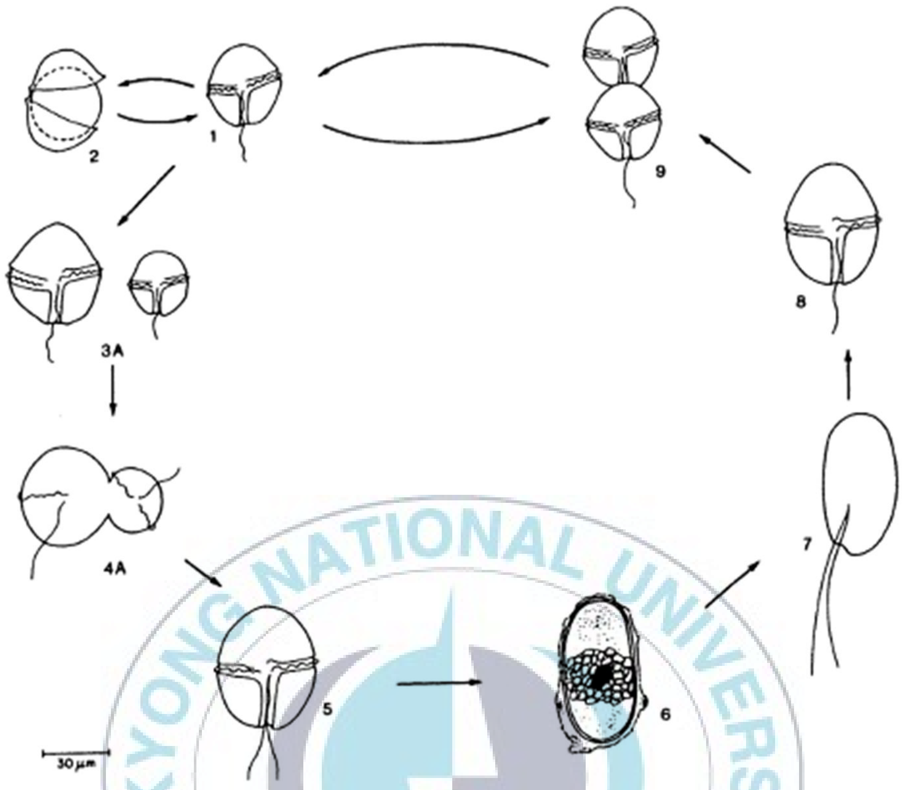

Fig. 1. Life cycle diagram of *Alexanderium tamarense.* Stages are identified as follows: (1) vegetative, motile cell; (2) temporary or pellicle cyst; (3) anisogmous "female" and "male" gametes; (4) fusing gametes; (5) swimming cell or planozygotes; (6) resting cyst or hypnozygotes; (7&8) motile, germinated cell or planomeiocyte; and (9) pair of vegetative cell following division. (from Anderson,1998)

Hypothesis is that the PSP development is closely related to cysts germination and vegetative cells number of *Alexandrium* spp. that affected by some physical factors (temperature and day length). Those physical factors are seasonally variable.

The purpose of the present study is to confirm the causative organism of PSP and the rhythm of *Alexandrium* spp. life cycle. To know important aspect of PSP potentials and to improve the relationships between the regional distribution of the resting cysts and the occurrences of *Alexandrium* spp.

2. Materials and Methods

2.1. Study site

Sediment samples for enumeration of cyst potential and cells development were collected bimonthly from four stations at Jindong-ri and three stations at Sujeong-ri in Jinhae Bay from January to May 2014. The latitude and longitude were determined continuously at the study site (Figs. 2-3).

2.2. Observation of physicochemical parameters

Water temperature, salinity, pH and 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).

Table 1. Location of sampling stations at Jindong-ri in Jinhae Bay from January to May 2014

Station	Latitude	Longitude
JD1	35º 09’ 76” N	128º 49’ 25” E
JD2	35º 08’ 84” N	128º 48’24” E
JD3	35º 09’ 38” N	128º 48’34” E
JD4	35º 09’ 19” N	128º 49’ 98” E

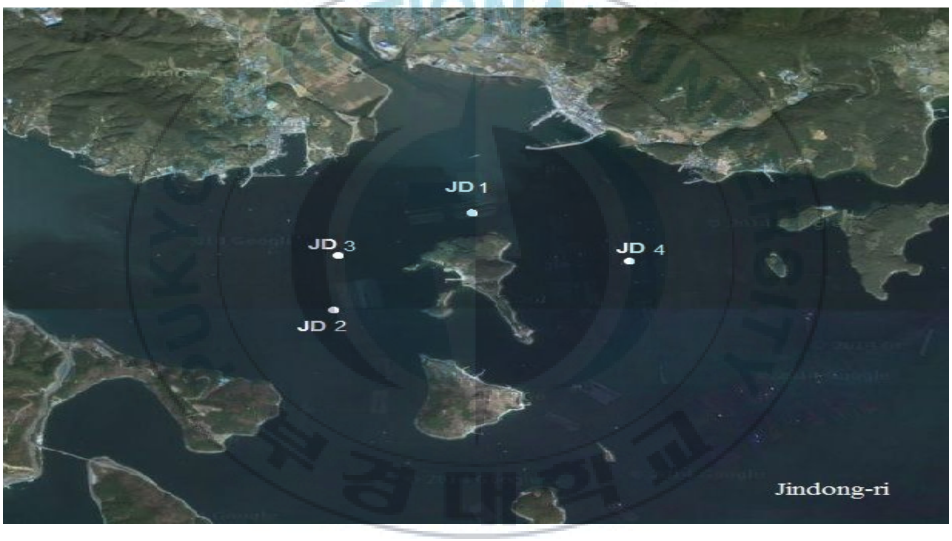

Fig. 2. The sampling stations at Jindong-ri in Jinhae Bay from January to May 2014.

Table 2. Location of sampling stations at Sujeong-ri in Jinhae Bay from January to May 2014

Station	Latitude	Longitude
SJ1	35º 12’ 32” N	128º 60’33” E
SJ2	35º 12’ 63” N	128º 60’86” E
SJ3	35º 12’ 91” N	128º 61’40” E

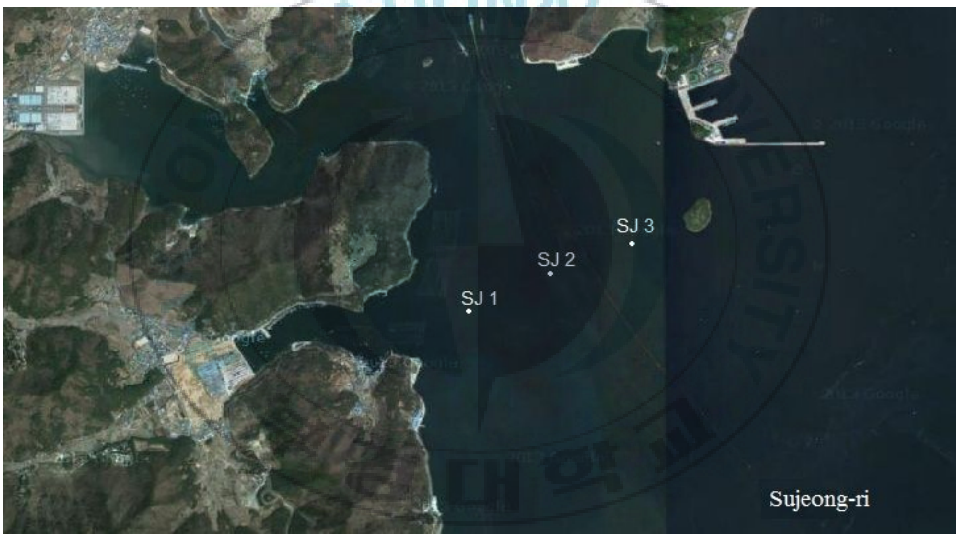

Fig. 3. The sampling station at Sujeong-ri in Jinhae Bay from January to May 2014.

2.3. Sediment samples treatment

Collected sediment were placed into small plastic bottles and stored at 4 ºC in dark place until they were used. Ten grams (wet weight) of each sediment samples were suspended in filtered sea water (FSW) and sieved though a nylon mesh to obtain the size fraction 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 counting chamber under the microscope equipped at 100X magnification.

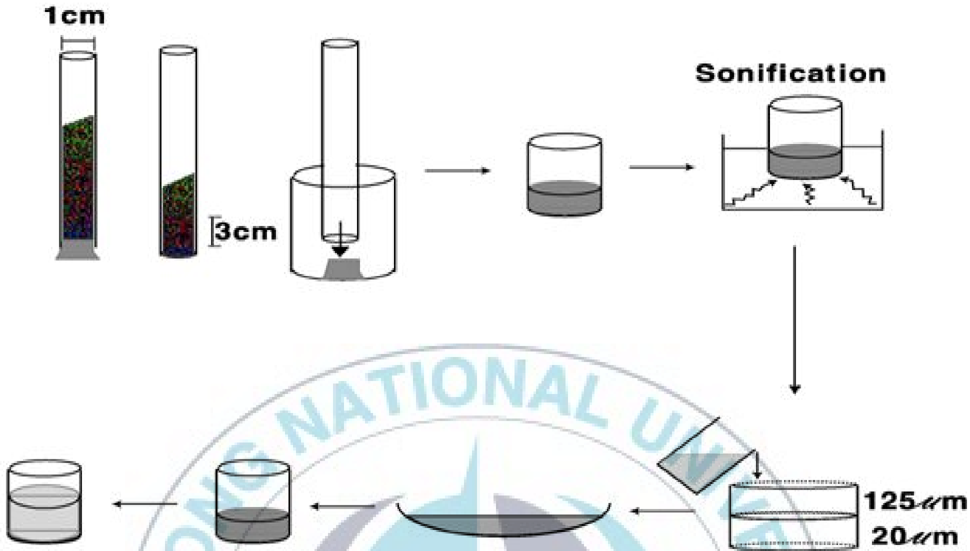

Fig. 4. Procedure for the cycle concentration from sediment samples (from Matzuoka and Fukuyo, 2000).

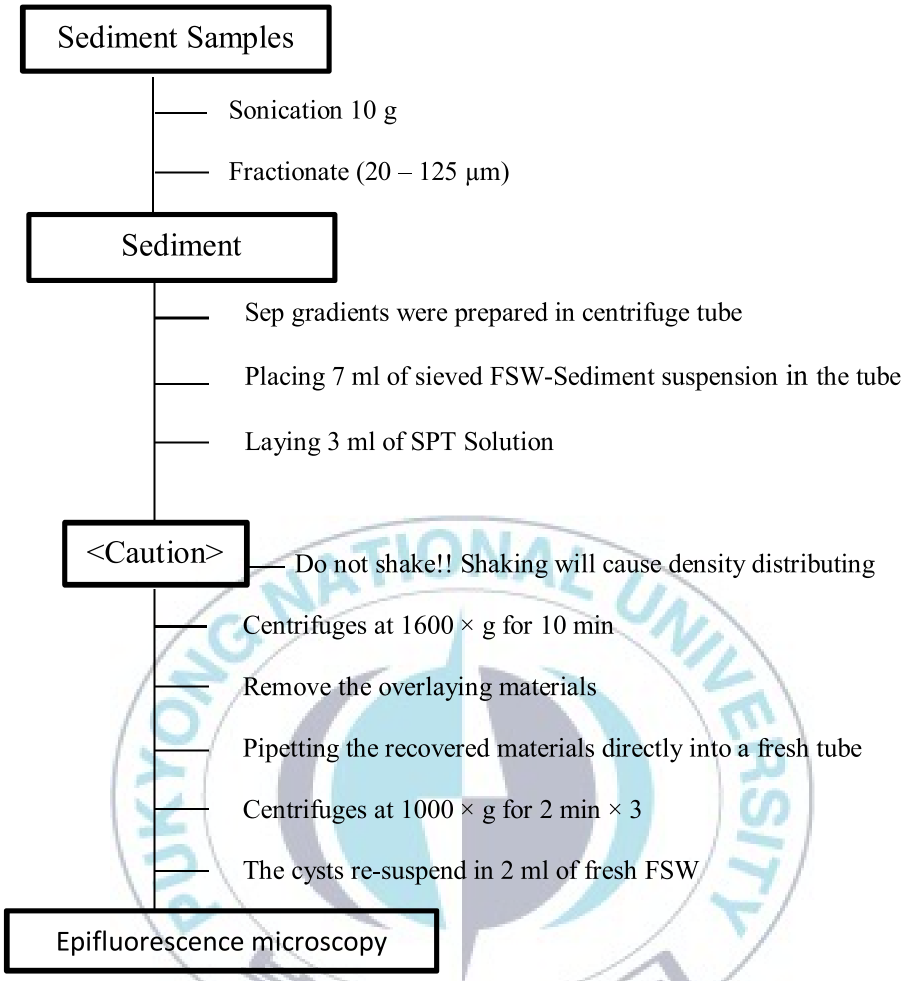

Fig. 5. Protocol for SPT (sodium polytungstate) treatment method for the enumeration of *Alexandrium* resting cysts in natural sediment. (from Matzuoka and Fukuyo, 2000).

2.4. Statistical analysis

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

3. Results

3.1. Spatio-temporal distribution of *Alexandrium* cysts

Forty-nine samples were collected during experiment period at 7 stations in Jinhae Bay from Jun 2014 to end of May 2014, *Alexandrium* spp. cysts were found in all the samples, but the abundance was variable from station to another the highest variable concentration rage of 33-117 cysts/cm^3 that's shown in distribution patterns (Figs .6-12).

The abundance of *Alexandrium* cysts is varied horizontally among the sampling stations in Jinhae Bay where the highest concentration was founded in SJ2 (65.6±35.7) and relatively higher distribution pattern showed around Sujung stations (Fig. 7; Table 3).

According to monthly abundance of *Alexandrium* cysts concentration was varied during the experiment period that showed the highest abundance concentration of *Alexandrium* cysts in January.

Table 3. The distribution of *Alexandrium* spp. cysts/cm^3 in sediment samples at Sujeong-ri and Jindong-ri in Jinhae Bay from January to May 2014

Stations	Date of sampling 2014						
	08-Jan	20-Jan	09-Mar	23-Mar	06-Apr	Apr19	18-May
SJ1	53	68	46	37	24	28	32
SJ2	113	117	63	59	33	39	35
SJ3	29	36	26	25	20	21	25
JD1	60	67	54	60	37	41	43
JD2	44	59	35	37	28	32	33
JD3	31	55	30	28	17	25	22
JD4	52	57	36	32	30	31	30
Total	382	459	290	278	189	217	220

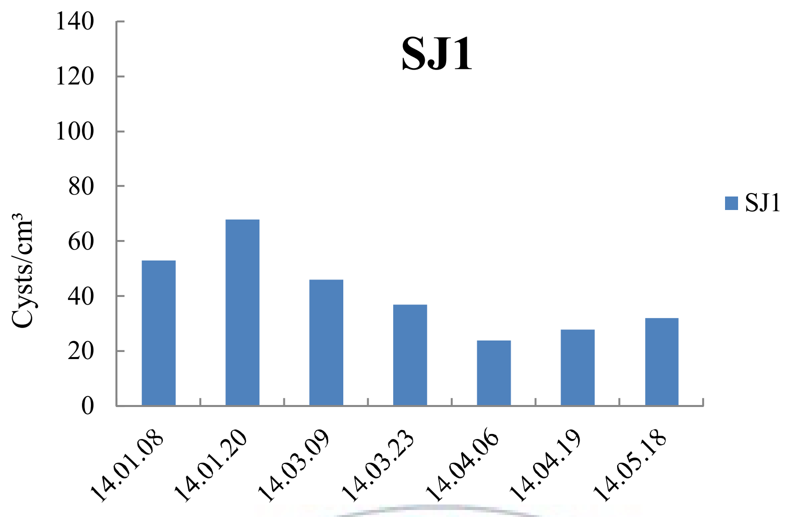

Fig. 6. Monthly variation abundance of the *Alexandrium* spp. cysts in the sampling sediment at SJ1 station in Jinhae Bay from January to May 2014.

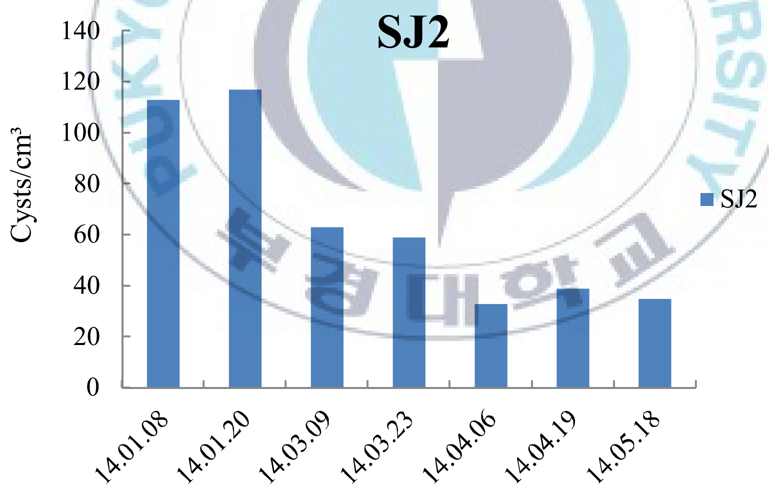

Fig. 7. Monthly variation abundance of the *Alexandrium* spp. cysts in the sampling sediment at SJ2 station in Jinhae Bay from January to May 2014.

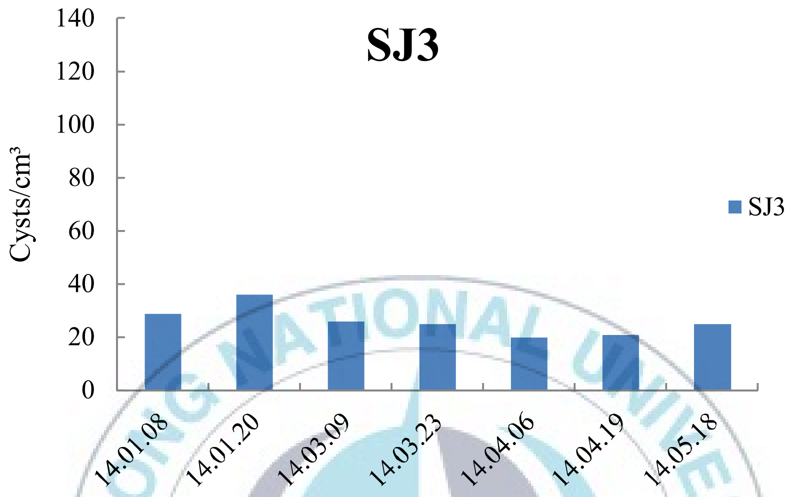

Fig. 8. Monthly variation abundance of the *Alexandrium* spp. cysts in the sampling sediment at SJ3 station in Jinhae Bay from January to May 2014.

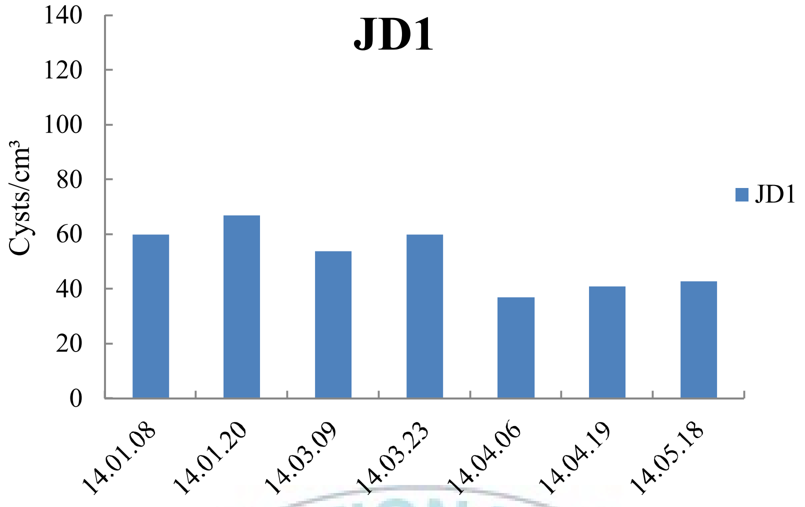

Fig. 9. Monthly variation abundance of the *Alexandrium* spp. cysts in the sampling sediment at JD1 station in Jinhae Bay from January to May 2014.

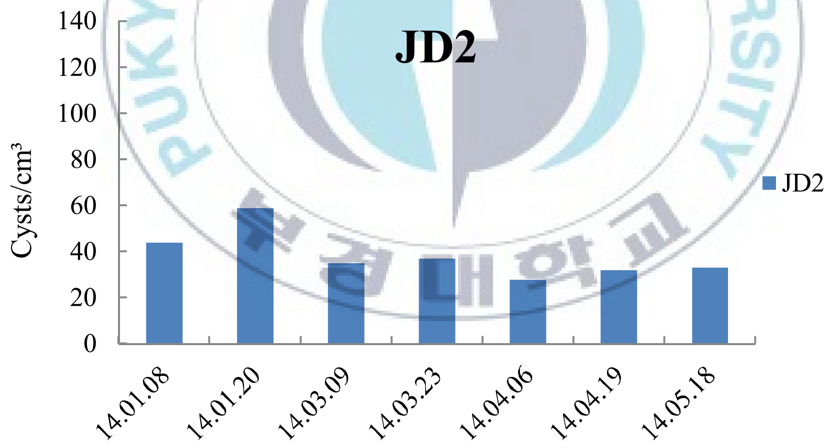

Fig. 10. Monthly variation abundance of the *Alexandrium* spp. cysts in the sampling sediment at JD2 station in Jinhae Bay from January to May 2014.

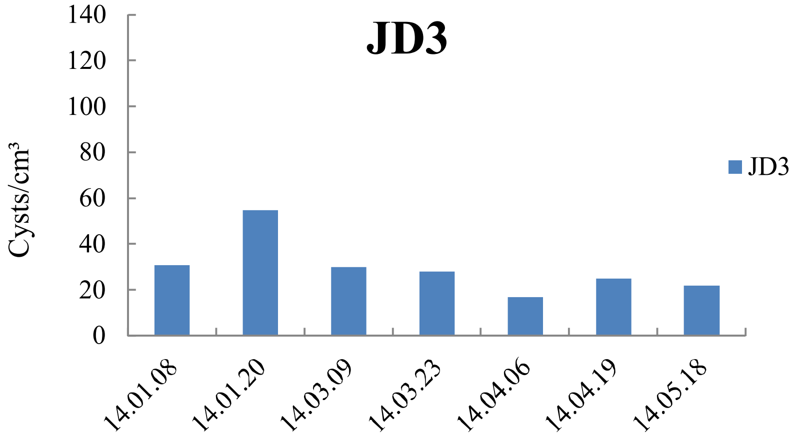

Fig.11. Monthly variation abundance of the *Alexandrium* spp. cysts in the sampling sediment at JD3 station in Jinhae Bay from January to May 2014.

Fig. 12. Monthly variation abundance of the *Alexandrium* spp. cysts in the sampling sediment at JD4 station in Jinhae Bay from January to May 2014.

Table 4. Results of SPSS test for the abundance of *Alexandrium* spp. cysts/cm³ among the station in sediment samples during sampling from January to May 2014

Dependent variable: Cyst abundance/cm³ at stations			
Stations	N	Mean	SD
SJ1	7	41.1	15.6
SJ2	7	65.6	35.7
SJ3	7	26.0	5.4
JD1	7	51.7	11.4
JD2	7	38.3	10.4
JD3	7	29.7	12.2
JD4	7	38.3	11.4
Total	49	41.5	13.4

Sum of Squares	df	Mean Square	F	P
6940.286	6	1156.7	3.7	0.005
13105.714	42	312.0		
20046	48			
R^2= 0.7	P= 0.001	N= number of samples		

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

The motile cells of *Alexandrium* spp. were appeared in samples in Jinhae Bay at both stations Jindong and Sujung, there was a cross relationship between the abundance *Alexandrium* cysts and cells. The motile cells of *Alexandrium* ssp. were founded in both of surface and bottom sea water at all stations in Jinhae Bay during the survived period. There was a significant variation between the stations, the vegetative cells densities were at beginning of March (9.3±2.6 cells/ml) then reached at top at the end of April (131.7±78.6 cells/ml) (Fig. 13-19, Table 5-6).

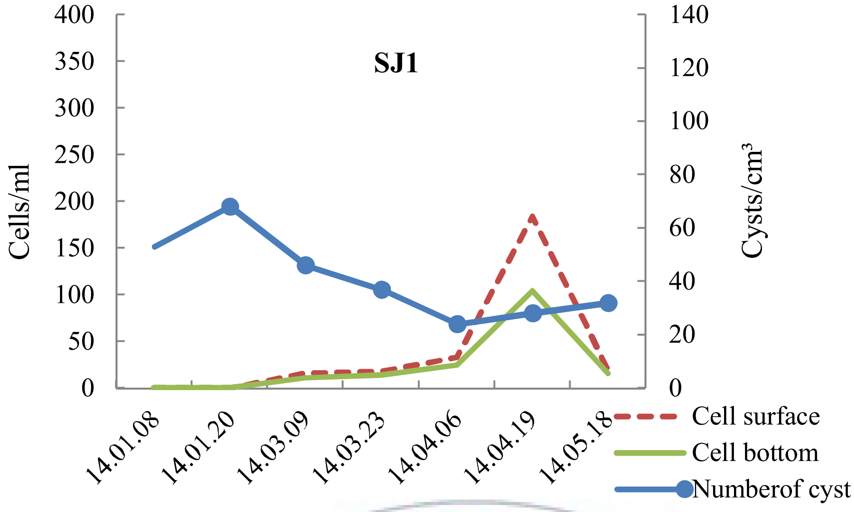

Fig. 13. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at SJ1 station in Jinhae Bay from January to May 2014.

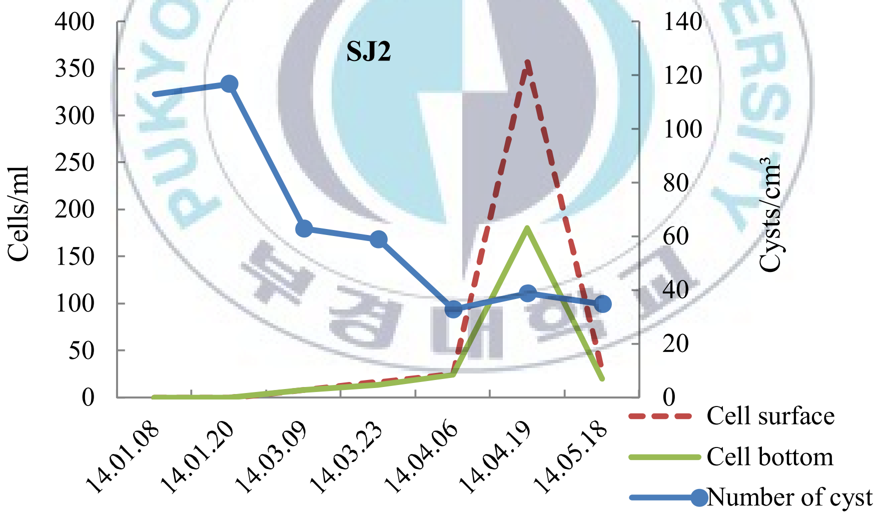

Fig. 14. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at SJ2 station in Jinhae Bay from January to May 2014.

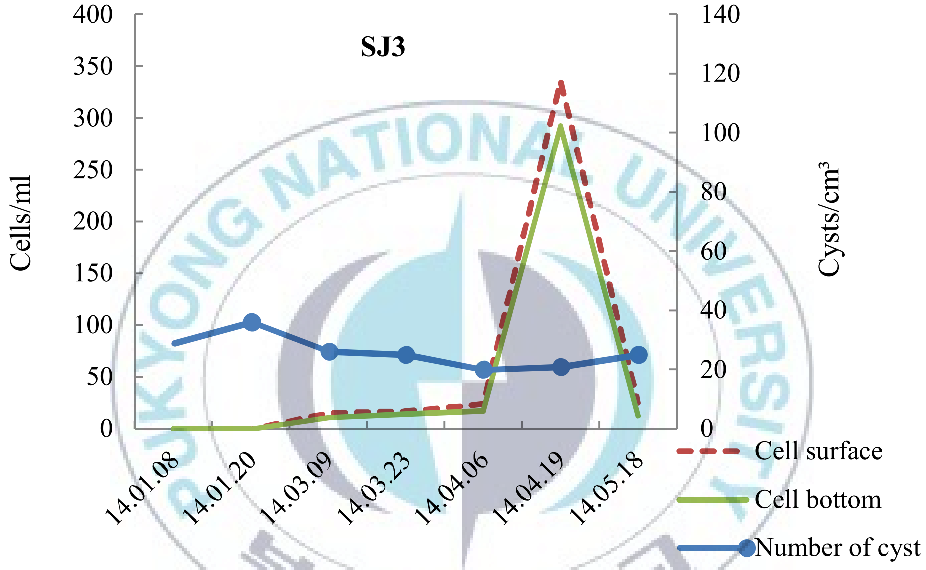

Fig. 15. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at SJ3 station in Jinhae Bay from January to May 2014.

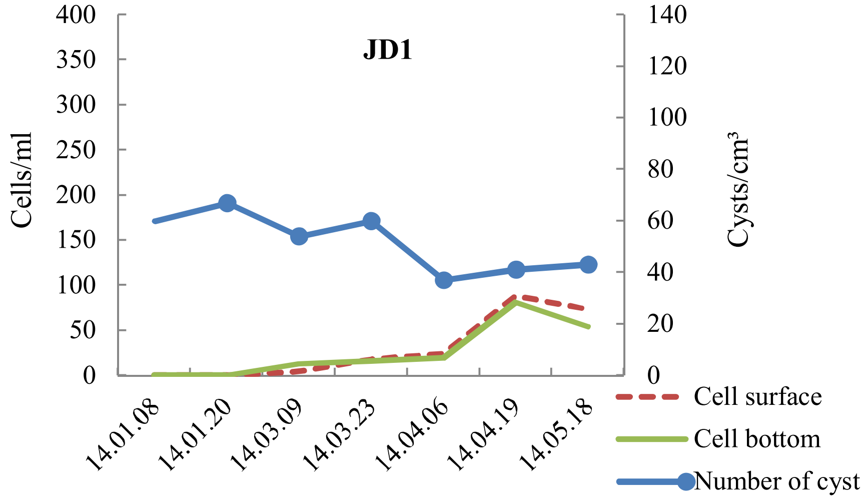

Fig. 16. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at JD1 station in Jinhae Bay from January to May 2014.

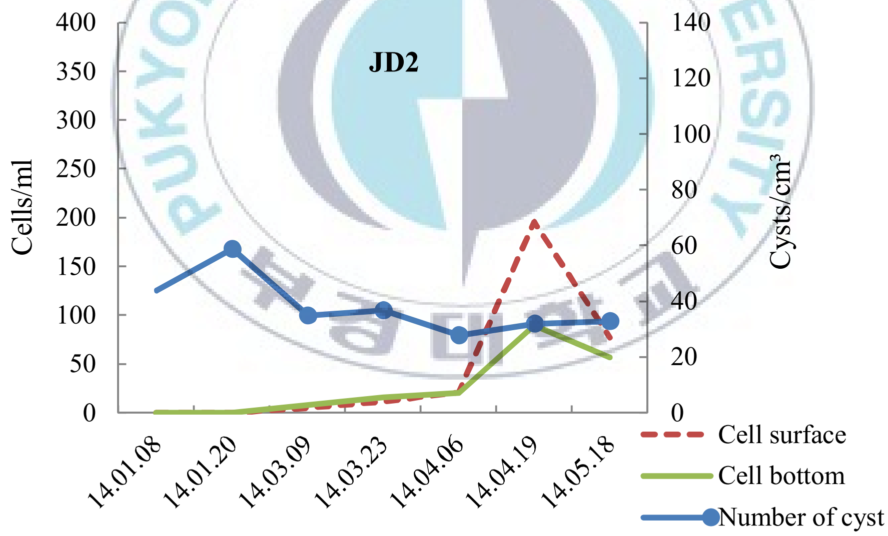

Fig. 17. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at JD2 station in Jinhae Bay from January to May 2014.

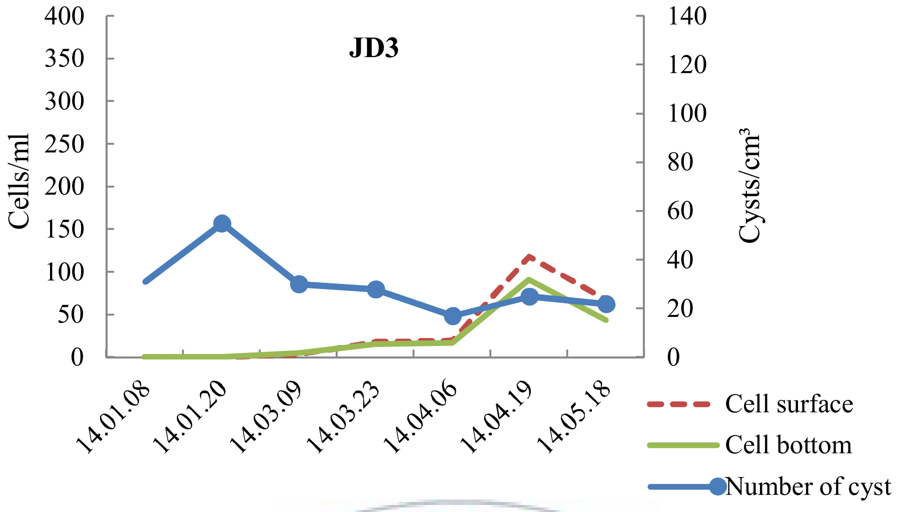

Fig. 18. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at JD3 station in Jinhae Bay from January to May 2014.

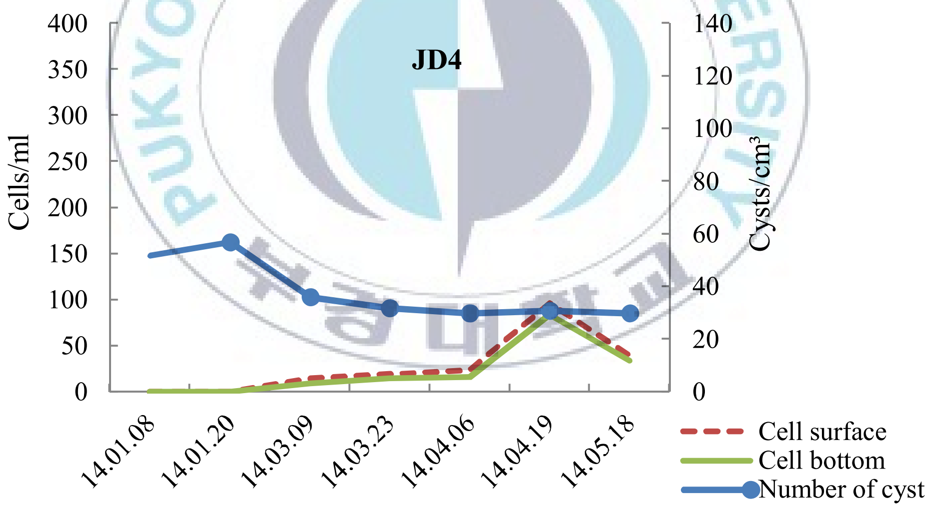

Fig. 19. Monthly variation of vegetative cells and cysts of *Alexandrium* spp. at JD4 station in Jinhae Bay from January to May 2014.

Table 5. Results of SPSS test one-way (ANOVA) for the abundance of *Alexandrium* spp. cells/ml among the station in Jinhae Bay from Jan to May 2014

Dependent variable: Abundance of cell/ml 2014				
Date	N	Mean	SD	P-value
09-Mar	7	9.3	4.2	
23-Mar	7	15.8	2.2	
06-Apr	7	22.0	4.4	0.000
19-Apr	7	164.1	98.3	
18-May	7	40.6	21.9	

*N=number of samples

SPSS analysis variance shown a significant differences ($P < 0.05$) in the cell abundance at all stations from Jan to May 2014.

Table 6. ANOVA test of the abundance of *Alexandrium* spp. cells in surface water among all the stations in Jinhae Bay form Jan to May 2014

Dependent variable: The number of surface cells 2014				
Stations	N	Mean	SD	P-value
SJ1	5	38.7	128.1	
SJ2	5	62.4	35.6	
SJ3	5	59.6	71.6	
JD1	5	29.6	43.9	0.000
JD2	5	44.4	34.4	
JD3	5	32.4	3.5	
JD4	5	27.3	3.5	

*N=number of samples

ANOVA analysis showed significant difference (P>0.05) the abundance of surface cells among the stations during survey periods.

3.3. The phytoplankton population and vegetative cells appearance during survey periods

The phytoplankton population was enumerated at tow stations (SJ2 & JD) Sujeoug-ri and Jindong-ri in Jinhae Bay from January to end of April 2014. The population was variable in both stations in during experiment period; the highest phytoplankton population was founded at JD3 station (79865± 4138.69 cells/ml) in January 2014 (Fig. 20, Table 7). The number of phytoplankton in the surface water was more than bottom water at both stations SJ2 and JD3 (Fig. 20-21).

The vegetative cell number shown cross relationship with phytoplankton population, when the phytoplankton population was high in January and February the vegetative cells were few in the samples later in March the population of phytoplankton was dropped down and vegetative cells numbers increased at both stations in Jinhae Bay from January to end of April 2014 (Fig. 20-21).

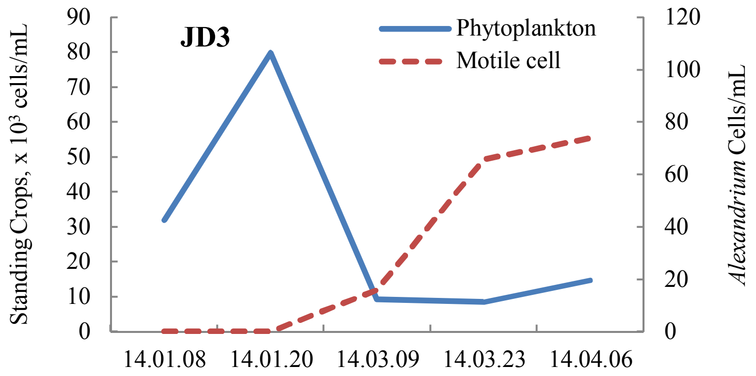

Fig. 20. Monthly variation of phytoplankton population standing crop and number of *Alexandrium* cells at Jindong-ri (JD3) station in Jinhae Bay from Jan to May 2014.

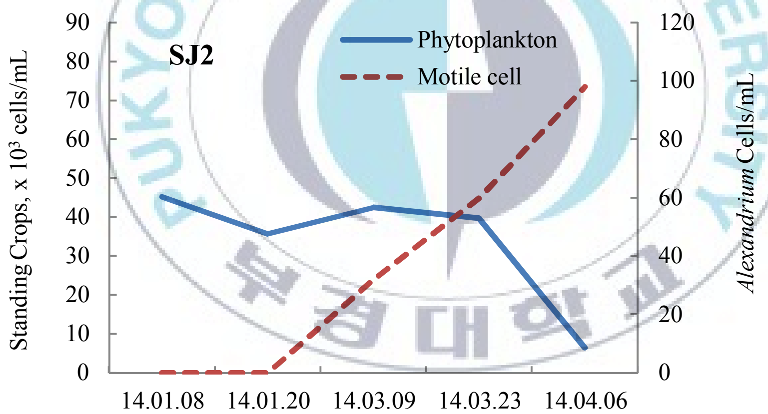

Fig. 21. Monthly variation of phytoplankton population standing crop and number of *Alexandrium* cells at Sujeoug-ri (SJ2) station in Jinhae Bay from Jan to April 2014.

Table 7. Results of SPSS test one-way (ANOVA) for the abundance of Phytoplankton population among the station in Jinhae Bay from January to end of April 2014

Dependent variable: The number of Phytoplankton cells				
Stations	N	Mean	SD	P-value
JD3	5	28869.6	30042.2	0.005
SJ2	5	33966.8	15717.6	

*N=number of samples

SPSS analysis variance shown a significant differences (P <0.05) in the phytoplankton population at two stations from January to end of April 2014.

4. Discussion

The mapping of *Alexandrium* spp. cysts in sea sediments are useful to mark the 'hot spot' where PSP outbreak have occurred or where it will be occur in future (Tyler et al., 1982: Anderson and Keafer 1985). However, hot spot areas are important for monitoring the PSP. In Korea, since from the first PSP was reported in 1986, and (Lee et al., 1992) defined that the occurrence of *Alexandrium* was a causative organisms of PSP in the cold waters from January to April. Later Kim (1995) found the seasonality of *Alexandrium* resting cysts germinability that were collected from Jinhae Bay, and the vegetative cells appeared in the cold season could result from their benthic cysts germination which have controlled by seasonal rhythms rather than the general environmental factors such as temperature.

The occurrence of *Alexandrium* and the abundance of resting cysts has been monthly surveyed in Jinhae Bay since 1996. Usually vegetative cells were appeared in January and disappeared in May or June, and mostly their standing crop are shown in April, when the water temperature becomes increased. High intensity of swimming cells can be assigned by environmental factors such as water temperature, but the inoculum of swimming cells into water was resulted by resting cysts germination during two cold months. The appearance of swimming cells reaches at high levels after sudden decreased on cysts number. The germination of resting cysts determined by regional population and quantitative variation, the germination rate of the cysts were higher in January and February of the year. This means that the germination of cysts was controlled by endogenous biological clock. Those results were also reported by others (Anderson and Keafer 1987; Perez et al. 1998), instead of a little effecting by environmental factors such as temperature and day length.

In this study, the cysts and vegetative cells of *Alexandrium* spp. were enumerated at 7 stations in Jinhae Bay from January to May 2014, the highest number of cysts were founded at station SJ2 station 117 cysts/cm³ and SJ3 around Sujeong-ri stations, and the lowest cysts numbers are shown in Jindong-ri stations exactly at JD3 station, 55 cysts /cm³ (Figs. 6-12, Table 3). Compared to 2012 (Ibrahim 2013) at the same stations, Sujeong-ri stations showed the lowest number of cysts 54 cyst/cm³ and the Jindong-ri station were highest number of cysts, 121 cysts/cm³. Moreover, the swimming cells that mostly appeared in January and reach high intensity in March or April, and then disappeared in May or June, in 2014 were the cells shown a little late in their appearance. That was also the reason for late standing crop in the middle and end of April. The number of vegetative cells were varied from surface to bottom of sea water, the intensity of cells were high on surface and less on the bottom of sea water, the same as reported by Shin, 2002 and Shon, 2005. The PSP levels were 42-60 µm/100g in Jinhae Bay during the end of March and April 2014, In Jangseug-po the PSP levels were 42-318 µm/100g at the same period reported (NRFDI, 2014), as known the quarantine level is up to 80 µg/100 g. The low levels of PSP in Jinhae Bay during spring 2014 were related directly to the lowest number of cyst germination at all stations in Jinhae Bay was noted by (Kim 1994; Kim and Lee 1996). In the other hand, the phytoplankton population was differed according to the period and location in Jinhae Bay from January to April 2014, two stations in Jinhae Bay were surveyed one Jindong-ri and another Sujeong-ri from January to April 2014. Both were phytoplankton population enumerated and shown the high intensity in January then slightly decreased in April 2014, But the highest standing crop was at Jindong-ri station.

Marine environment conditions can be assessed by changes in total cyst productivity and comparative changes in a particular taxon (Dale et al., 1999; 2002, Matsuoka. 1999; Cho et al., 2011). In the Global aspect *Alexandrium* was distributed and its incidence in the 1990s was over twice considerable to those of 1970s (Anderson 1994). The increase was due to coastal pollution and shipping movement (Carlton 1985; Hallegraeff et al. 1988).

PSP toxification enormously usually happened in spring, at the same time with vegetative cells appearance of these species, but the accumulation of toxin in shellfish may be resulted by several appearance of vegetative cells during the year and reach maximum toxin levels in April.

5. Conclusion

I studied Spatial Distribution of Resting Cysts and Vegetative Cells of PSP Toxigenic *Alexandrium* spp. in Jinhae Bay, Korea

The study revealed that the cysts germination is responsible of vegetative cells density during warming period and that determined the PSP development. The number of cysts and cells intensity was highest at Sujeong-ri St. 2 in Jinhae Bay during the survey.

The timing and location of algal blooms can depend on the cysts potentials and its germination, and where they are deposited.

The density of vegetative (motile) cells elevated in April and the cysts number dropped down at the same time. It means that the benthic cysts out germinate to inoculate motile cells in water column.

Alexandrium spp. has frequently appeared for the last two decades in Jinhae Bay. for this reason it's helpful for carrying out a study there and compared to previous researches in order to better understand *Alexandrium* spp. of which life cycle is affected by some environmental factors. And then to find ideal solution for PSP problems in the future.

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