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# Harmful Dinoflagellates and Mitigation Strategies in Korea

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**Harmful algal blooms (HABs) have been responsible for numerous fish kills and public health problem. Since 1995, massive *C. polykrikoides*-related fish kills and *Alexandrium* spp.-related shellfish poising from Korean waters. have been recorded To address this issue, geographic distribution of harmful algae, molecular methods for identifying harmful algae, HABs mitigation strategies, impact on some fisheries animals, life cycles, and toxicity have been studied to understand their bloom dynamics. The paper is a review on the studies of harmful dinoflagellates and mitigation.**

Key Words: bloom dynamics, *Cochlodinium polykrikoides,* Harmful algal blooms, mitigation,

## INTRODUCTION

Mariculture for finfish or shellfish in the coasts of Korea is popular. Many pen cages, particularly for finfish culture, are mostly concentrated along the southern coast where there are many embayments enabling aquaculture facilities safe from typhoons. However, harmful algal blooms (HABs) have been increasing year by year since 1980s due to eutrophication caused by industrialization and increase of aquaculture sites. Moreover, HABs by *Cochlodinium polykrikoides* occurring in both inshore and offshore have been prevailing since the 1990s resulting in severe fisheries damage in the area coasts (Han et al. 1993; Kim et al. 1999; Lee et al. 2001; Kim et al. 2001). Accordingly, HABs by microalgae have gained increasing attention over the last three decades in Korea.

It has been known that the harmful alga, *C. polykrikoides*, blooms normally from August to September annually since the1990s in Korean coasts leading to fish kills by suffocation with oxygen depletion rather than any toxic substances itself (Kim et al. 1999; Kim et al. 2000; Kim et al. 2001; Kim et al. 2002). The first fisheries damage by a HAB (1.7 million US dollars) in the Korean coast occurred in 1981 and was caused by *Karenia mikimotoi.* Thereafter, *C. polykrikoides* blooms have brought about mass mortality for cultured finfish almost every year in Korean coasts since 1993. Particularly, there was huge harmful algal blooms in 1995, resulting in about 95 million dollars' fisheries damage (Kim 1998; Kang et al. 2002).

Yellow clay dispersion has been applied to minimize fisheries damages by *C. polykrikoides* blooms since 1996. The practical field application of the mitigation techniques by yellow clay played a great role in reducing fisheries damages in Korea.

On the other hand, toxic *Alexandrium* species, largely *A. tamarense,* that cause 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 & 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

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would be beneficial for the early prediction and warning of HAB occurrence or intoxification of bivalves.

This report documents the overall research outcomes related to the bloom dynamics, monitoring including results on life history of *C. polykrikoides*, and the mitigation techniques by yellow clay application for harmful algae in Korea.

## Harmful dinoflagellates in Korea

### HAB events, major causative species and seasonality

The data on HAB events have been based on monitoring/ base field studies done monthly over 92 regular stations by National Fisheries Research and Development Institute (NFRDI) on weekly and/or biweekly base HAB outbreak report by extension service as a monitoring program in Korea, which includes details on HAB events, causative organisms, maximal cell density, location, water temperature, etc.

A total of 525 algal bloom events (of which 153 are HABs in Korean coasts during 1999-2008, as shown in Fig. 1). The annual HAB events showed gradual decrease (i.e., 47-84 events in 1999-2004 and 28-39 events in 2005-2008, respectively). The HAB events caused by two fish killing algae (*C. polykrikoides* and *Chattonella* spp.) increased at the same period of time (13-40% in 1999-2004 to 26-73% in 2005-2008). The principal taxonomic groups were dinoflagellates rather than diatoms (Fig. 2). The major HAB species was *C. polykrikoides* (140 events*), Heterosigma akashiwo* (62), *Prorocentrum dentatum* (52), *Noctiluca scintillans* (35), *Akashiwo sanguinea* (30), *Prorocentrum minimum* (21) and *P. triestinum* (17) in dinoflagellates and *Skeletonema costatum* (35), *Thalassiosira* spp. (13). and *Chaetoceros* spp. (11) in diatom during the period. Although the blooms by non-fish killing species occurred in closed and/or semi-enclosed embayment such as Jinhae Bay, Buksinman, Ulsanman, etc., blooms by fish killing species, *C. polykrikoides*, occurred in offshore and inshore areas except in the closed embayment where nutritional level is relatively high.

HABs by dinoflagellates and diatoms have been observed almost all the year round irrespective of the season (Fig. 3). About 90% of HAB during 1999-2008 appeared from May to September with the highest peak occurrence during high water temperature season from June to September (82%). The majority of the events during the high water temperature season, particularly from August to September, were attributed to the *C. polykrikoides* blooms.

### Status of *C. polykrikoides* bloom and fisheries damage

*C. polykrikoides* bloom has been mostly terminated from mid-September to mid-October since it made initial outbreaks from mid-July to mid-August when coastal

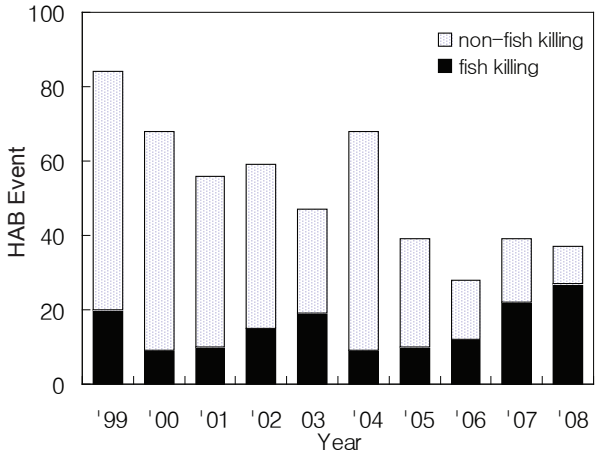


**Figure 1**. Number of Harmful Algal Blooms events in Korean coasts during 1999-2008 (Source: Annual reports of harmful algal blooms in Korean coastal waters published by NFRDI).

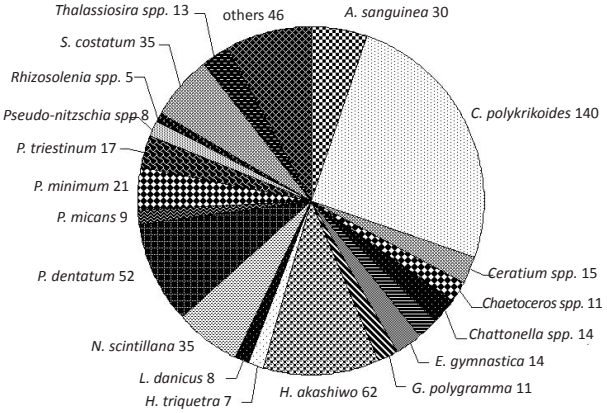


**Figure 2**. Major Harmful Algal Blooms causative species in Korean coasts during 1999-2008. A total of 563 species has been responsible for HABs during the period (Source: Annual reports of harmful algal blooms in Korean coastal waters published by NFRDI).

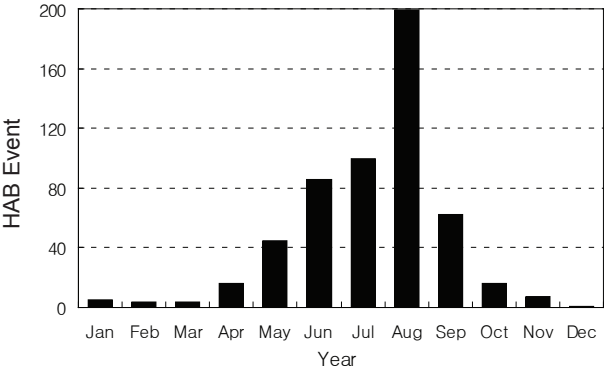


**Figure 3**. Monthly average variation of Harmful Algal Blooms events in Korean coast during 1999-2008 (Source: Annual reports of harmful algal blooms in Korean coastal waters published by NFRDI).

**Table 1**. Status of *Cochlodinium polykrikoides* bloom in Korea during 1999-2008.

| | '99 | '00 | '01 | '02 | '03 | '04 | '05 | '06 | '07 | '08 |
|---|---|---|---|---|---|---|---|---|---|---|
| Initiation | Aug.11 | Aug.22 | Aug.14 | Aug.2 | Aug.13 | Aug.5 | Jul.19 | Aug.6 | Jul.31 | Jul.30 |
| Termination | Oct. 3 | Sep.19 | Sep.24 | Sep.27 | Oct.13 | Sep.3 | Sep.14 | Oct.30 | Sep.18 | Sep.29 |
| Duration (d) | 54 | 29 | 41 | 57 | 62 | 30 | 58 | 36 | 50 | 62 |
| Affected area | South, East | South, East | South, East | South, East | South, East | South | South | South | South, East | South, East |
| Max. density (cells/mL) | 44,000 | 15,000 | 32,000 | 30,000 | 48,000 | 5,800 | 25,000 | 22,500 | 32,500 | 7,300 |
| Fish kills (thou. ind.) | 248 | 258 | 7,557 | 5,268 | 13,088 | 219 | 1,638 | 702 | 9,570 | 0 |
| Econ. loss (billion won) | 0.32 | 0.26 | 8.4 | 4.9 | 21.5 | 0.12 | 1.06 | 0.07 | 11.5 | 0 |

water temperature elevated to 24-25°. *C. polykrikoides* bloom lasted for 29-62 days until termination, showing maximal cell density of 7,300-48,000 cells/mL (Table 1). The affected area by *C. polykrikoides* bloom was both in the southern coast of Korean peninsula and in East Sea/Japan Sea except from 2004 to 2006 when the blooms were only restricted in the southern coast without spreading to eastern coasts.

The first fisheries damage occurred by harmful algae, *Karenia mikimotoi* in 1981 in Korea. Thereafter, *C. polykrikoides* bloom has brought about mass mortality of finfish and shellfish almost every year since 1993. Particularly, there was huge harmful algal blooms in 1995, resulting in about 95 million US dollars' fisheries damage. The biggest fish kills by a *C. polykrikoides* bloom during 1999-2008 occurred in 2003 with 13,088 individuals of killed cultured finfish and shellfish, equivalent to 21.5 billion wons of economic loss. Considering the bloom strength calculated based on maximal cell density, duration and affected area, the bigger fisheries damage occurred when the bloom strength was relatively higher (Fig. 4).

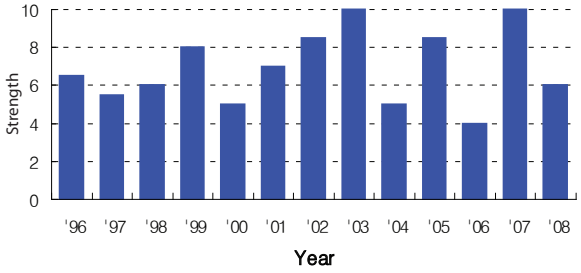


**Figure 4**. The variation of relative bloom strength by *Cochlodinium polykrikoides* in Korea during 1996-2008. The relative bloom strength was calculated by giving each 0-3 points for three parameters (maximal cell density, bloom scale and duration) and 0-1 points for fisheries damages considering their strength (Source: Annual reports of harmful algal blooms in Korean coastal waters published by NFRDI).

## HAB mitigation strategies

Korean yellow clay composed of montmorillonite has been used to control HABs, particularly targeting *Cochlodinium* blooms since 1996. The fisheries damage in 2003 (22.3 million dollars) was caused by the mass mortality in land-based abalone culture farm. However, the decrease of annual fisheries damage on finfish has been remarkable since the field application of yellow clay beginning1996.

Several devices such as clay dispenser, automatic HAB alarm system, and special ship equipped with the system of electrolytic clay dispenser have been developed for fisherman and/or HAB responsible agencies, enabling early HAB early warning or effective HAB control. Clay dispenser, enabling wild yellow clay to crumble into fine size less than 100 μm to maximize HAB removal rate and automatically dispense into the HAB affected area, has been developed and deployed to HAB control responsible agencies since 1997 (Fig. 5). A special device, Electrolytic Clay Dispenser (ECD) combined generator (for seawater electrolysis with clay dispenser) has been developed and deployed to the local government since 2001 in Korea (Fig. 5). The device has been aimed at both maximizing HAB removal efficiency and minimizing the amount of clay used for control to reduce the amount of clay to as much as 70% and increase HAB removal rate as high as 95%. This new device has been more beneficial than old style of clay dispenser. Also, an automatic HAB alarm system equipped with detection sensor for chlorophyll and turbidity has been developed and propagated to each private land-based finfish culture farm since 1999 so that fishermen can get warning sound or signals from the

**Figure 5**. Harmful Algal Blooms control by Clay dispenser (left) and electrolytic clay dispenser (right) in Korea.

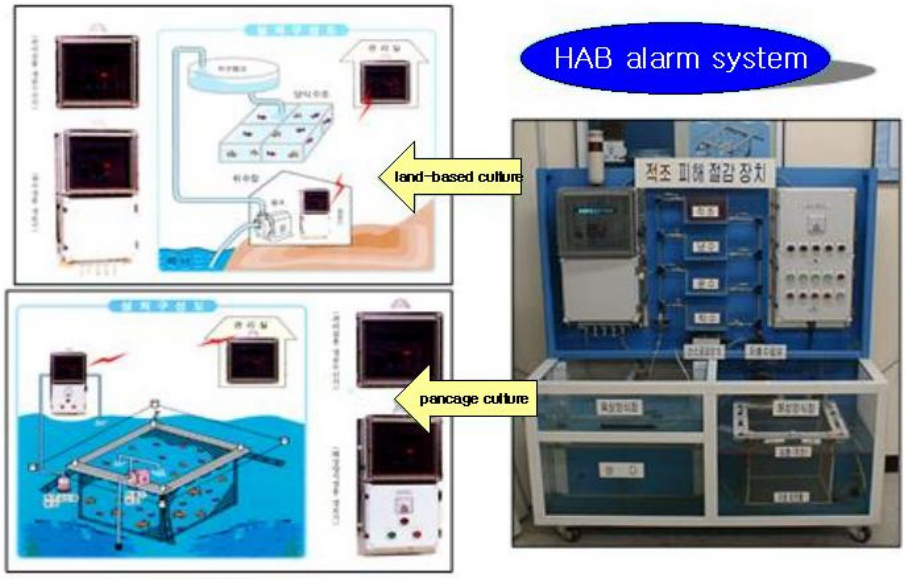


**Figure 6**. Harmful Algal Blooms alarm system notifying fisherman of HAB appearance by alarm sound.

device whenever red tide is present in their aquaculture sites (Fig. 6).

### Impact on fisheries animals

Laboratory experiment was conducted to test the impact of clay on aquatic animals by monitoring either the respiration rate or clearance rate when aquatic animals were exposed to different concentrations of clay suspensions (0.05%, 0.25%, 1.25%, 6%; wt./wt.).

The results showed that the amount of clay in gills and cavities of mussel (*Mytilus galloprovincialis*), oyster (*Crassostrea gigas*), and abalone (*Haliotis discus hannai*) increased rapidly over time and the animals became filled with clay within 2 h after exposure to different clay suspensions (0.05%, 0.25%, 1.25%; wt./wt). Depuration of the accumulated clay from shellfish was evident within 30 min following removal from clay suspensions and was discharged completely within 6 h (Fig. 7). After a peak in Clearance Rate (CR) at 30 min, values declined quickly and became equivalent to the untreated control groups within 2 h, although clay particles were still present in shellfish (mussel and oyster) tissues. These results did not reveal an obvious impact of clay concentration on CR for oyster or mussel excluding abalone whose respiration rate showed drastic decline even at the low clay concentrations of 0.05%.

Oxygen consumption by rockfish (*Sebastes schlegeli*) was affected minimally by the lowest clay concentrations (0.05%, 0.25%, 1.25%; wt./wt.). Oxygen consumption rates increased slightly with exposure to clay suspensions

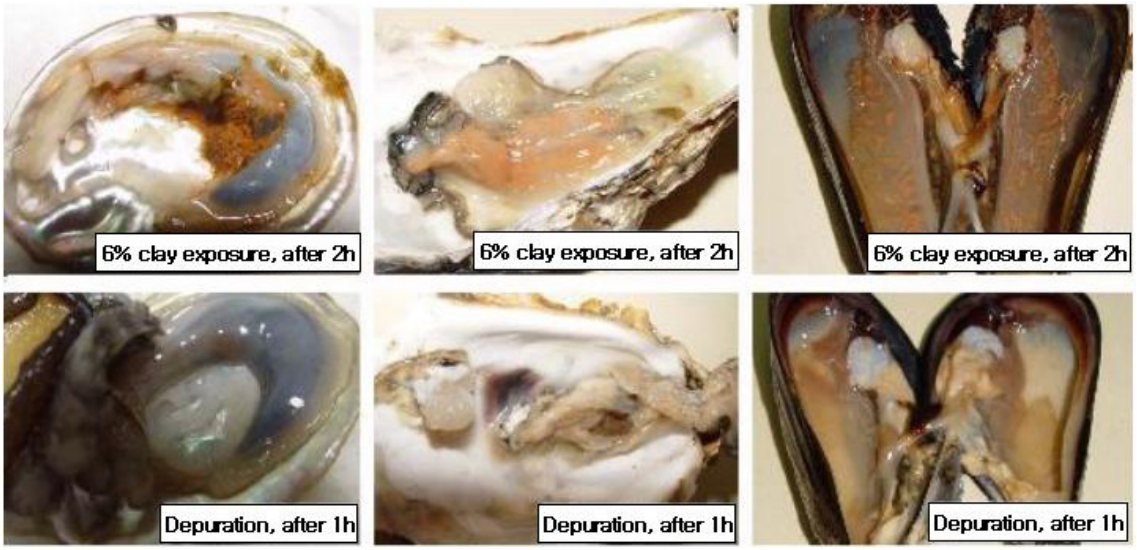


**Figure 7**. Accumulation of clay in the cavities of shellfish (left to right: abalone, oyster, and mussel) in 2 hours after exposure to 6% of clay suspensions (upper) and depuration of clay from their cavities in one hour after removal from clay water (lower).

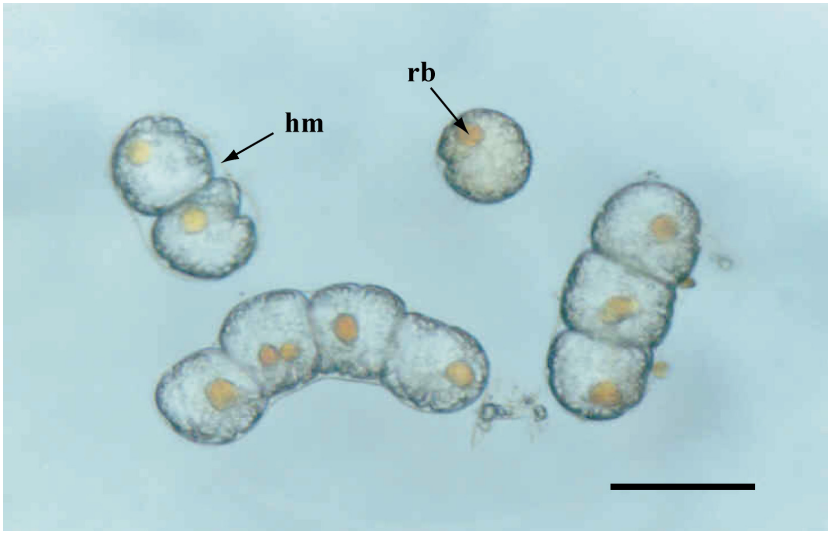


**Figure 8**. Chains of hyaline cysts of *Cochlodinium polykrikoides*. rb, red body; hm, thin hyaline membrane. Scale bar = 40 μm.

but recovered to previous levels immediately thereafter. Given that field applications of yellow clay in Korea are generally < 0.04% (V/V) and the clay particle is easily dispersed into open water by tides and currents, the effect of clay dispersion on endogenous biological rhythms of rockfish (e.g., oxygen consumption) might be negligible.

### Bloom of *Cochlodinium polykrikoides*, with emphasis on life cycle elucidation

The characteristics of the initial *Cochlodinium polykrikoides* bloom outbreak have promoted a hypothesis that the organism is transported to Narodo area via an offshore, marine current. However, there are a number of unsolved questions concerning this hypothesis. In addition, little is known about the species life cycle, except for the planktonic unarmored vegetative stage (Kim et al. 2002) and the armored types which germinated from resting cysts (Kim et al. 2007) found during the bloom-forming period.

### Hyaline cysts formation and regeneration

While studying *C. polykrikoides* blooms along the Korean coast, hyaline cysts were found being produced by this species. The hyaline cysts were immobile, pale in color,

and nearly lacked chloroplasts. Their size was similar to that of the motile cells. Only faint traces of the sulcus and the cingulum were present on the cyst surface. All hyaline cysts were surrounded by a transparent, thin hyaline membrane (Fig. 8). After being preserved for six months at 4ºC in darkness, *C. polykrikoides* cells regenerated successfully from the hyaline cysts when moved into the light and a higher temperature. Individual motile cells regenerated one by one from the chain of hyaline cysts (Kim et al. 2002). The hyaline cysts can be considered a kind of temporary cyst.

**The role of resting cysts as discreate regional point sources on bloom initiation**

A common assumption is that cyst "seedbeds" provide the inoculum for many harmful blooms. Given the widespread cyst distribution typical of coastal areas, one important scenario is that it could be possible that the blooms are initiated by the synchronized germination of cysts throughout the region. In fact, good examples of discrete cyst seedbeds that lead to large-scale blooms do exist along the northern shore of the St. Lawrence estuary (Cembella et al. 1987).

To investigate the development of armored cells into more normal vegetative cells morphologically, as seen during the initial bloom-forming period, an armored type isolated from Saemangeum (SMG11) was cultured for one year. The culture was then exposed to room temperatures (20-25°C) in August, assuming that the armored type would be a planomeiocyte of *C. polykrikoides*. Addition of f/2-Si medium to the culture caused active cell divisions, but development into an unarmored vegetative cell occurred rarely, except for a single morphological change into four-cell chain unarmored vegetative cells within two days (Kim et al. 2007).

Therefore, we investigated the cyst distribution along the coastal areas such as Gosung-Jaran Bays and Wando, where *Cochlodinium* blooms were prevailing in 2007. As expected, a high distribution of cysts which is presumed to be *Cochlodinium* cysts was shown only in some stations of the coastal areas where blooms occurred last year (10-15 cysts/10 g sediments in wet weight) and stations where a physical accumulation is possible (98 cysts/10 g sediments), while the other stations showed just a trace of their distribution (0-2 cysts/10 g sediments). On the other hand, from their feature that the cyst population germinate under favorable environmental conditions, it is estimated that their densely populated regions take the role of point sources of recurrent bloom initiation in the shallow coastal areas (Kim CH, personal communication). Therefore, 1) a precise investigation of cyst distribution should be done in the red tide frequent areas; 2) the best way to prevent red tide would be to find the measure which could remove the bloom potential in the areas where cysts are abundant. The quantitative dynamics of the species' cyst populations clearly requires further studies.

**Quantification of *Cochlodinium polykrikoides* in surface and sediment samples using real-time PCR**

Vegetative cells of *C. polykrikoides* are morphologically defined by their distinct characteristics of the cingulum and are identified by light microscopy and/or scanning electron microscopy (SEM) on cultured strains (Matsuoka et al. 2008). For identification of dinoflagellate cysts, light microscopy and SEM observations are also required, and the field-derived cysts are commonly incubated in culture media over a period of several weeks for growth and then the germinated cells are re-examined by microscopy for species verification. These conventional methods require considerable time and expertise for species identification. To overcome this difficulty, molecular identification methods such as real-time PCR assay, fluorescent *in situ* hybridization, and sandwich hybridization have been used for detection of harmful dinoflagellates. Of these approaches, real-time PCR assay has been used for field-based studies on phytoplankton (Bowers et al. 2006; Lin et al. 2006; Park et al. 2007a, b).

Real-time PCR is a technique that provides highly sensitive, quantitative, and rapid detection of dinoflagellates. It is a closed-tube format that eliminates post-PCR processing and enables high analysis. Real-time PCR technologies can be grouped into amplicon sequence non-specific and sequence specific methods (Mackay et al. 2002). Amplicon sequence specific methods include TaqMan, Molecular beacons, and Scorpion PCR (Monis & Giglio 2006; Tomlinson et al. 2007). Real-time PCR, incorporating fluorogenic 5′ nuclease (TaqMan) chemistry, has been used for the detection and enumeration of a number of dinoflagellates in environmental samples (Moorthi et al. 2006; Kamikawa et al. 2007; Park et al. 2007a). Of amplicon sequence non-specific methods, SYBR Green I is currently the industry standard and other intercalating dyes such as BEBO, LC Green, and SYTO9 have been also used (Bengtsson et al. 2003; Monis et al. 2005). The SYBR Green I system has been used to detect dinoflagellate DNA in mixed algal cultures and environmental samples (Galluzzi et al. 2004; Lin et al. 2006). Intercalating dyes are cost efficient compared with sequence-based fluorogenic probes, and these allow confirmation of PCR amplicons by DNA melting curve analysis. Since these dyes detect any double-strand DNA molecules, the formation of non-specific amplicons must be checked by melting curve analysis for species-specific detection. SYTO9 chemistry has been known to provide melting curve analysis performance superior to that of SYBR Green I (Monis et al. 2005; Monis & Giglio 2006), and it produces highly reproducible melting curves over

a broader range of dye concentrations and amounts of starting DNA template (Monis et al. 2005). The SYTO9 method has been also recently applied to the dinoflagellate detection and a field-based study (Park et al. 2009a).

Many dinoflagellate species have been reported to form resting cysts or other types of resting stages as part of their life cycle (Anderson & Keafer 1987). The germination of overwintering cysts of dinoflagellates such as *Alexandrium* spp. provides the "seed population" for harmful algal blooms (e.g., Anderson 1980). The life cycle proposed for *C. polykrikoides* includes vegetative stages, a temporary cyst (as hyaline cyst), and three different morphotypes of resting cysts (Matsuoka & Fukuyo 2000; Kim et al. 2002, 2007). The formation of hyaline cysts or resting cysts may play an important role in initiation of *C. polykrikoides* blooms (Kim et al. 2002). A recent study developed a TaqMan based real-time PCR probe to the ITS rDNA region for enumeration of *C. polykrikoides* abundance in surface and sediment samples (Park et al. 2009b). The real-time PCR probe was used for investigation of the geographic distribution and abundances of *C. polykrikoides* and the morphologically similar dinoflagellate *Gymnodinium impudicum* in Korean waters and sediments (Park et al. 2009b unpublished data ). The field survey from June to September 2007 showed that *C. polykrikoides* cell densities peaked in the South Sea in August at 16,928 cells $mL^{-1}$, and similar cell densities were also obtained by using microscopic cell counts (Park et al. 2009b). The assays were also successfully used to quantify *C. polykrikoides* cysts in the sediment, and showed a positive association between distribution of *C. polykrikoides* in sediments and its presence in past water samples (T.G. Park, unpublished data ) indicating that "seed beds" of *C. polykrikoides* may exist in Korean sediments and the germination of dormant cysts provides the "seed population" for initiation of *C. polykrikoides* blooms.

**Paralytic Shellfish Toxin (PST) production of *Alexandrium tamarense/catenella* complex from southern coastal and offshore waters of Korea**

Two PSP incidents that resulted in human deaths were reported from Korea in 1986 and 1996. Chang et al. (1997) first reported a correlation between the occurrence of *A. tamarense* (as *Protogonyaulax tamarensis*) and shellfish intoxication, and Kim (1995) demonstrated that the causative microorganisms were *A. tamarense* in Jinhae Bay, Korea. A number of studies have been performed over the last two decades to verify the toxin production of PSP causative organisms. Also, the PSP toxin composition of single *Alexandrium* isolates is generally considered stable throughout the broad range of physico-chemical conditions. Moreover, PSP toxin composition has been used as a practical biomarker to differentiate among *Alexandrium* isolates from different geographical locations (Kim et al. 1993; Park et al. 2004). Therefore, we established toxic *Alexandrium catenella* and *A. tamarense* isolates from the southern coastal and offshore waters in Korea, and analyzed the PSP toxins to elucidate any differences in toxin production related to locality.

*A. tamarense* and *A. catenella* from coastal areas contained carbamate toxins as the major toxin component, and were clearly distinguishable from those from offshore areas that contained *N*-sulfocarbamoyl toxins as the major toxin component. This variation can be attributed to differences in environmental regimes. A perpetual front is located between the two areas and prevents the direct influx of the Tsushima Current into coastal areas. Thus, the environmental differences between these two areas may result in their distinctive geographical populations. Similarly, Park et al. (2004) reported that *Gymnodinium catenatum* strains in the Yellow Sea at a higher latitude compared with the south inshore of Korea included isolates producing higher toxicity with carbamate toxins predominantly. Furthermore, it was suggested that high toxicity of the northern isolates was due to their production of the highly potent carbamate toxins, and the regional trend in toxicity could result from latitudinal differences in environmental parameters and their influence on the establishment of the genotypically different blooms (Anderson et al. 1994).

From the cluster analysis, isolates from the Southern offshore belonged largely to Group I, while isolates from the southeastern sea including Jinhae Bay belonged mostly to Group II-III. Moreover, there is also a strong possibility that a variety of toxin components in regional populations found in Jinhae bay isolates resulted from the genetic exchange in sexual reproduction of phenotyphically different toxin profiles (Sako et al. 1992; Ishida et al. 1993). Therefore, this might be explained by heterogeneity with a genetic trait as well as with the advantage of environmental separation (Cembella et al. 1987; Kim 1995; Park et al. 2004) rather than by the influence of the regional environments, such as elevated nutrients (Group III are dominant in Jinhae Bay) and compositional changes observed in culture studies (Hwang & Lu 2000).

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