Harmful Algae News

An IOC Newsletter on Toxic Algae and Algal Blooms

No. 46 - June 2012 · www.ioc-unesco.org/hab

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

HAB Research and Management in Korea

Overview of HAB research and controls

Outbreaks of harmful algal blooms (HABs) in Korea were sporadic until the 1980s, but became frequent during the 1990s. During the last three decades, HABs in Korean coastal waters have led to deleterious effects such as risks to human health, loss of natural and cultured seafood resources, constraints on tourism and recreational activities, and damage to natural aesthetics and biodiversity. Of major concern are mass fish mortalities and shellfish poisoning syndromes. In August 1981, Korea experienced the first mass mortalities of fish and shellfish caused by *Karenia mikimotoi* blooms in Chinhae Bay. In 1995, a fish killing dinoflagellate, *Cochlodinium polykrikoides*, caused widespread and persistent blooms accompanying the worst economic loss of US$ 95.5million. PSP has also been a troublesome issue for marine food safety since the 1980s.

Since then, Korea has established comprehensive national HAB monitoring including shellfish toxins for early warning and management. Clay dispersal to control fish killing *C. polykrikoides* blooms began in 1996 (Fig. 1). In recent years, HAB research activities are mostly conducted by national institutes, universities, and government supported institutes. The on-going HAB projects are nationwide HAB monitoring, bio-optics and remote sensing, population dynamics, toxin chemistry and toxicity, benthic harmful algae, impacts of HABs, management and mitigation, and awareness and capacity building. Some on-going HAB studies are introduced here.

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Fig. 1. Aerial view of merry-go-round clay dispersal off Yokjido Is on south coast of Korea, to control fish-killing Cochlodinium *bloom approaching fish cages, August 21, 2008, photographed by Jong-Suk Jung. Source : Hak Gyoon Kim, Pukyong National University, hgkim@nfrdi.go.kr*

National HAB Monitoring and prediction system

HAB monitoring covering all Korean coasts has been conducted by national institutes since 1996. NFRDI (National Fisheries Research and Development Institute) is primarily in charge of routine red-tide monitoring and conducts monthly HAB surveys at 98 sites in Korean coastal waters, from March to November, to investigate monthly changes of water quality and dominant phytoplankton species. More than 30 fisheries extension service stations under local governments are also responsible for irregular HAB monitoring, whenever water discoloration occurs within or in adjacent waters. In addition, once HABs by fish-killing species are initiated, NMPA (National Maritime Police Agency) also join the red-tide monitoring program and conduct aerial surveillance with helicopter. In addition, NFRDI has conducted a specific monitoring program targeted on *Cochlodinium* blooms since 2000. This monitoring primarily aims at early prediction and warning of *Cochlodinium* blooms based on biological and hydrological data collected in biweekly field surveys from late June to early October. To sustain safe supplies of shellfish products without shellfish poisoning, NFRDI, in collaboration with local fisheries extension services, conducts regular monitoring of oyster, mussel and clam farms. More than 100 sites are monitored monthly from February to November with special emphasis on the eastern part of South Coast where oyster farms are concentrated. When shellfish poisoning is detected, NFRDI increases monitoring frequency from monthly to weekly.

The HAB clearing house in NFRDI collects all available biological and hydrological data from red-tide monitoring institutes and coastal environment observation buoys deployed near aquaculture farms, and meteorological and remote sensing data, to synthesize, analyze and publish a daily red-tide newsletter. The daily HAB newsletter describes causative organisms, cell densities, and affected areas, and provides warnings and predictions on the development and spread of red-tides: it is disseminated to fisherman and relevant agencies in charge of red-tide monitoring and/or mitigation by ARS (Automated telephone Response System), SMS, Satellite TV, easy fax and internet webpage (www.nfrdi.re.kr).

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National marine biotoxins monitoring and responses

Contamination of bivalves with marine biotoxins such as paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) and other lipophilic shellfish toxins (LST) are controversial issues in Korea. Korea is a major producer of bivalves, and Korean shellfish products are exported to the US, China, Japan, EU and elsewhere (Fig. 2). The occurrence of marine biotoxins in Korean shellfish is considered to be important both in Korea and importing countries. The Marine Biotoxin Monitoring Program in Korea began in 1980, and was initially based on the screening of mussels for the presence of PSP toxins in shellfish growing areas on the south coast. Since the 1980s, 44 cases of PSP, of which 5 were fatal, were reported from the south coast of Korea, and all those who died had consumed toxic wild mussels. On the other hand, neither ASP nor DSP incidents have been officially reported in Korea. Since the 1990s, the PSP monitoring area was expanded to the east and west coasts of Korea, and DSP and ASP monitoring added to the program, which entails the participation of the central government, local governments and fisheries cooperatives.

Marine biotoxins are analysed at the National Fisheries Research and Development Institute, Busan. Local governments act to prevent shellfish poisoning caused by marine biotoxins, for example by closing harvesting areas. At present, PSP is measured by the mouse bioassay, and ASP and lipophilic toxins are analyzed using instruments such as HPLC or LC-MS/MS. Official regulatory limits for biotoxins in shellfish flesh are 800 $\mu g \cdot kg^{-1}$ for PSP; 160 $\mu g \cdot kg^{-1}$ for okadaic acid plus dinophysistoxin^{-1}, and 20 $mg \cdot kg^{-1}$ for ASP. When concentrations exceed the regulatory limit, shellfish harvesting areas are temporarily closed; recreational harvesting is also prohibited. PSP has occurred in the spring season, mainly March to May, on the southeast coast of Korea. Jinhae Bay is especially affected, and some areas in the eastern part of the bay exceed regulatory limits every year. Jinhae Bay is a typical semi-enclosed coastal area surrounded by cities and is one of the most eutrophic coastal embayments in Korea. This bay is known for recurrent red-tides including *Alexandrium tamarense* blooms. The eutrophic condition in Jinhae Bay is considered a major factor promoting the growth of toxic phytoplankton such as *Dinophysis* spp. or *Alexandrium* spp. Marine biotoxin occurrences, especially PST, begin in Jinhae Bay and expand to the southeast coast of Korea each year. LSTs in bivalves show remarkable seasonal and annual variations. Some sporadic LST occurrences were observed by mouse bioassay until 2008 when the LC-MS/MS technique was adopted and seasonal variation of LST occurrence could be investigated. Seasonal trends show that high LST values are a spring-summer problem. The major LSTs in Korean bivalves are okadaic acid and dinophysistoxin^{-1}, but occasionally pectenotoxin^{-2} or yessotoxin are detected as a major component. However, the toxicity in whole bivalves tissue has not exceeded the regulatory limit (160-200 µg · kg-1) in Korea, the EU, and the US. ASP is rarely found in Korean shellfish by HPLC analysis, and no poisoning incidents have been recorded.

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Fig. 2. Oyster farms in south Sea of Korea. Oyster production is one of the most important industries. Source : Ji Hoe Kim, Food Safety Research Division, National Fisheries Research and Development Institute, kimjh@nfrdi.go.kr.

Ocean-color remote sensing for HAB monitoring

The National Fisheries Research & Development Institute (NFRDI) has been using satellite remote sensing techniques since the 1990's to provide fisheries information in Korea (Fig. 3). The environmental parameters provided by satellites were initially limited to Sea Surface Temperature (SST) and water turbidity. After the launch of ocean color sensor SeaWiFs in 1997, chlorophyll concentration was considered a key parameter for monitoring red tides and water quality in Korean waters. But the satellite red tide information is corrupted by suspended sediment particles, especially in coastal waters, and remote sensing cannot be used for operational purposes. Moreover, the spatial resolution of SeaWiFS is not adequate to monitor coastal red tide patches. In the early 2000's, Korea Ocean Research & Development Institute (Dr. Y H Ahn, KORDI) carried out the basic research on red tide detection techniques, including the optical properties of phytoplankton, the modeling of ocean color changes by living organisms in the ocean, and inverse techniques of ocean colour. We anticipate that this research will be used in the near future hyper-spectral satellite (like HiCO) to detect and identify the

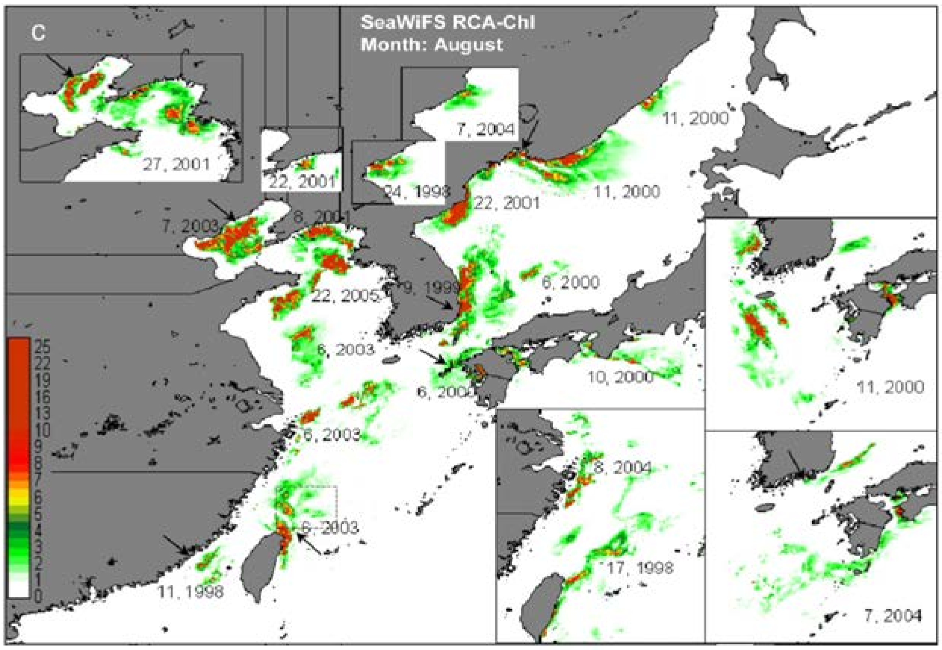

Fig. 3. The spatio-temporal distributions of the SeaWiFS detected red-tide by the Red-tide Chlorophyll Algorithm (RCA) in and around Korean waters during August 1998 – 2006. The bloom dates and years are mentioned in the figure. Source : Yu-Hwan Ahn, Korea Ocean Satellite Center in KORDI, yhahn@kordi.re.kr.

different phytoplankton groups from space. In addition, a new red tide algorithm developed by KORDI for ocean color satellite permits partial separation of chlorophyll from suspended sediment signals. But red tide detection techniques are not yet perfect, and continue to be an ongoing task for ocean colour scientists.

The new technique was applied in northeast Asian waters to the spatial distribution, movement and frequency of red tides. It revealed that most Korean coastal red tides do not develop in situ, but are advected from southern Taiwan. When they enter Korean waters with the current, the causative species meet nutrient rich waters and bloom again. These results indicate that Korean red tides start in the open sea rather than in coastal water. Since 2000, KORDI scientists have studied phytoplankton fluorescence signal (FS) of chlorophyll. The intensity of FS is proportional to chlorophyll concentrations in ocean waters and is not distorted too much by suspended sediment particles. In 2010, the first geostationary ocean colour Imager (GOCI, one of 3 payloads in the COMS satellite), mission developed by KORDI, adopting FS bands, was launched with the support of the Ministry of Land Transportation & Maritime Affairs (MLTM) in Korea. The main mission of GOCI is the monitoring of Korean coastal waters. Red tide monitoring is one of key missions using FS detection. Korea Ocean Satellite Center (KOSC in KORDI) is responsible for the data service and operating of GOCI, and receives images eight times a day. The Korea Aero-Space Research Institute (KARI) controls the COMS satellite. At the 15th ICHA, we will present the activities of satellite red tide monitoring and research results in Korea.

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Study of benthic dinoflagellates and monitoring

Biogeographically, Jeju Island is in the temperate zone based on air temperatures, but some tropical fish and invertebrates have been found here in the last decade. The Tsushima Warm Current, a branch of the Kuroshio Current, with high water temperature and high salinity, has influenced the sea around Jeju Island more strongly than before. We can assume that this island is changing from temperate to sub-tropical. We have found planktonic dinoflagellate evidence of the shift from temperate to sub-tropical, due to strengthening of the Tsushima Warm Current. The ciguatera agent *Gambierdiscus* sp. has been reported recently in Jeju waters. Until now, no toxic events caused by dinoflagellates have been reported; detailed information on the taxonomy, seasonal distribution, diversity and molecular phylogeny of benthic toxic and non-toxic dinoflagellates from this Island is not yet available.

The present study of benthic dinoflagellates has been conducted since March 2011 with the following objectives: to provide an overview of their diversity and systematics, to identify and describe them for environmental monitoring, and to develop monoclonal cultures of strains from Jeju waters. Dinoflagellate samples were collected around Jeju Island and cultures established. Preliminary observations reveal 32 species belonging to 15 genera. Among them, 10 species are reported to be toxic in subtropical and tropical regions and 27 are new to Korean waters. The potentially toxic species include *Amphidinium carterae, A. operculatum, Coolia monotis, Gambierdiscus yasumotoi, G. toxicus, Ostreopsis ovata, O. lenticularis, Prorocentrum emerginatum, P. concavum,* and *P. mexicanum.* Species of *Amphidinium* and *Ostreopsis* are the most abundant, and large numbers of *Ostreopsis* were found in June 2011. *G. yasumotoi* was found in only one location (Hwasun) in June 2011 and *G. toxicus* in Humduk in October and November 2011. *P. concavum* oc-

Fig. 4. Coolia monotis *(left),* Ostreopsis ovata *(middle) and* Gambierdiscus toxicus-*like (right) specimens. These subtropical species of harmful algae have been identified around Jeju Island off southwest Korea (scale bar = 10 µm). Source: Joon-Baek Lee (Jeju National University) and Md. Mahfuzur Rahman Shah (Jeju Sea Grant College Program, Korea, jblee@jejunu.ac.kr).*

curred in Shewa in August 2011, and *P. mexicanum* in October 2011 in Hwasun. A total of 150 strains of benthic dinoflagellates were established in the laboratory. Some species like *A. carterae* and *A. operculatum* are being cultured in 300ml, 1 L, 2 L, 3L flasks and 20 L carboys to scale up and optimize culture conditions. Newly recorded species are identified so far based only on LM, and more detailed morphological and genetic study are needed to confirm identifications.

The existence of subtropical/tropical benthic dinoflagellates in Jeju waters may point towards a change from temperate to subtropical. We believe that the occurrence of these species is related to stronger flow of the Tsushima Warm Current. The presence of potentially toxic tropical dinoflagellates (*Coolia monotis, Ostreopsis ovata, Gambierdiscus yasumotoi, G. toxicus*) (Fig. 4) may indicate a risk of potential toxicity in Jeju waters. Studies continue to establish single cell cultures (small scale to mass culture), biochemical (fatty acid, antioxidant, pigment) and toxicity analyses of the cultured strains, taxonomic comparisons using morphometric and molecular phylogenetic analysis, and seasonal abundance and distribution patterns in Jeju waters.

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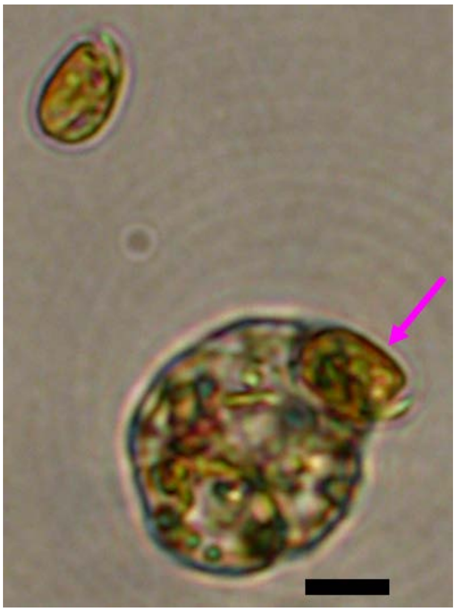

Fig. 5. The mixotrophic dinoflagellate Cochlodinium polykrikoides feeding on the dinoflagellate Amphidinium carterae (arrow). (scale bar = 5 µm). Source: Hae Jin Jeong, Seoul National University, hjjeong@snu.ac.kr; http://plaza.snu.ac.kr/~hjjeong/English.htm

Studies of planktonic food web and mixotrophy

Our research lines include i) interactions between harmful algae and their grazers or prey (Fig. 5); ii) marine planktonic food webs; iii) dynamics of C, N, P in marine ecosystems; iv) responses by the marine planktonic community to diverse scale environmental changes; v) mixotrophy in marine organisms; vi) horizontal gene transfer among marine protists; vii) invasive species, in particular, subtropical dinoflagellates (*Coolia, Gambierdiscus, Ostreopsis, Prorocentrum,* and *Amphidinium*); viii) *Pfiesteria* and similar species; ix) the taxonomy and eco-physiology of new dinoflagellates and ciliates (we established the dinoflagellates *Stoeckeria algicida, Luciella masanensis, L. atlantis, Paragymnodinium shiwhaense, Gyrodiniellum shiwhaense, Gyrodinium moestrupii* and the ciliates *Strombidinopsis jeokjo, Parastrombidinopsis shimii,* and *Balanion masanensis*). In addition, we are developing methods of controlling harmful algal blooms (i.e. mass-cultured grazers, NaOCl), reducing CO_2 using marine microalgae, and producing biodiesel using marine protists in food webs. For more information:

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Studies of marine phototrophic microbes (MPMs)

The main research interests of the MPM Lab in Kunsan National University are "strain-based" ecology and biotechnology of MPMs in Korean seas. MPMs include unicellular cyanobacteria, red tide plankton, and symbiotic eukaryotes such as zooxanthellae, parasitic dinoflagellates and epiphytic microalgae. Our study on MPM ecology includes the strain-based ecophysiology of *Amoebophrya* strains, *Mesodinium rubrum,* and *Dinophysis* spp. and the population ecology of bloom-forming MPM species in Korean seas. Successful isolation of *Teleaulax* sp. strains some 10 years ago was extended to setting up Korean *Mesodinium rubrum* strains, which initiated the novel establishment of *Dinophysis acuminata* strain DA-MAL01 in 2006. The availability of the three species in culture opened a new door for our research on the mechanisms for the blooming process, evolution of plastid and cell organelles, and even to multi-faceted biotechnological applications of previously "harmful" MPMs (Figs. 6, 7). At present, biotechnological aspects of MPMs strains in our lab are mostly related with *Dinophysis* spp. strains which have proved to be ideal provider organisms of the anti-cancer drug lead, pectenotoxin^{-2}(PTX^{-2}). Nitrogen-fixing unicellular cyanobacterium strains have been isolated and tested for the commercially reasonable production of hydrogen gas within 30 years in Korea.

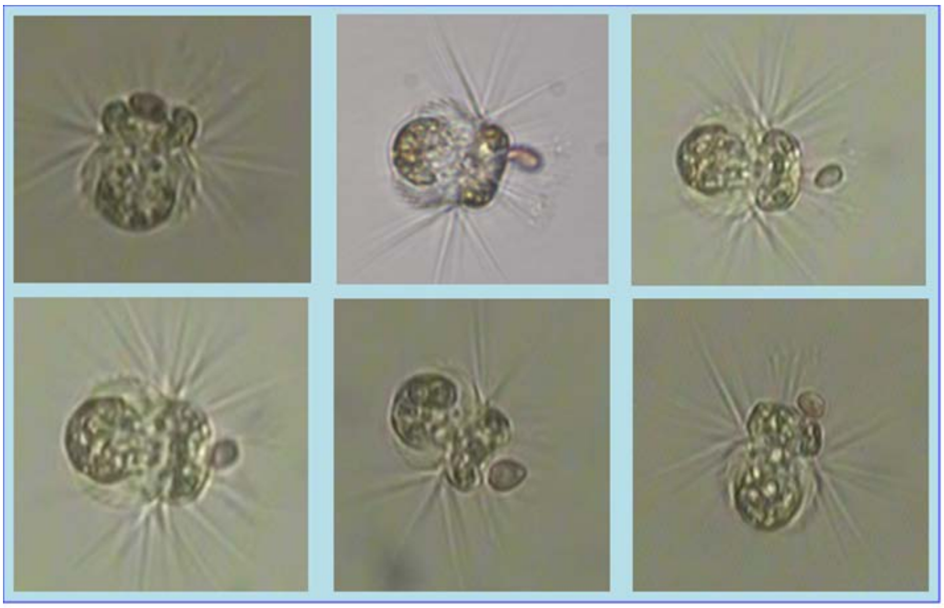

Fig. 6. From the phytoflagellate Teleaulax *to the ciliate* Mesodinium*, the first step of real-time plastid flow through food chain. Source: Wonho Yih, Kunsan National University, ywonho@kunsan.ac.kr*

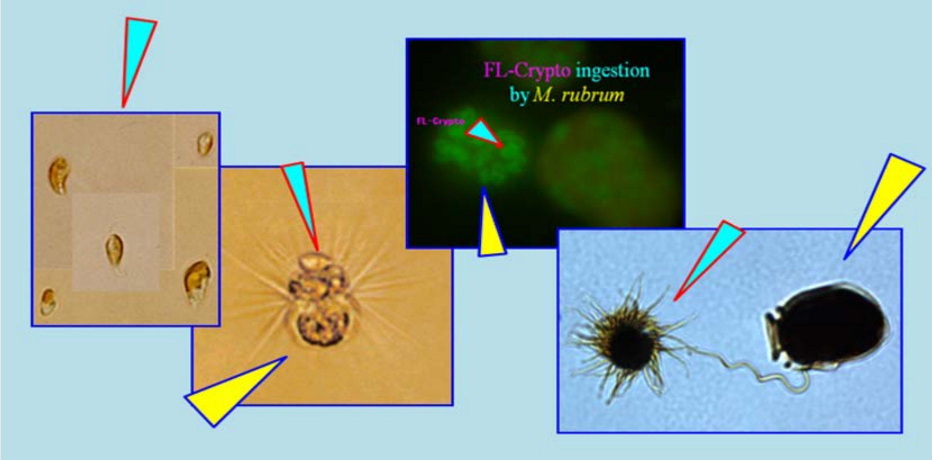

Fig. 7. From Teleaulax *through* Mesodinium *to* Dinophysis*. In a time scale of minutes to days, plastids of the phytoflagellate* Teleaulax *are obtained and reused serially more than two times by the ciliate grazer, dinoflagellate predator, and other even higher level consumers. Source: Wonho Yih, Kunsan National University, ywonho@kunsan.ac.kr*

Single cell (SC) physiology of cyanobacterial strains, such as growth synchrony, recycling of fixed carbon, and oxygen resistance of the hydrogen producing SC system, might be further studied. MPMs with diverse cell dimensions could also be developed as the promising live feed organisms for mariculture of marine animals, as the key organisms for the mobile CO_2 removing units, and also as sources of novel engineered enzymes, toxins, and pigments. In the near future, a kind of TBS (total biotechnology system) using MPM strains as the core could be designed and tested for economically and environmentally sound simultaneous production of multiple bio-products.

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Study of sophorolipid-yellow clay mixture to mitigate HABs

Numerous HAB mitigation methods have been examined in Korea, including yellow clay, marine bacteria, microscreen filtration, sodium hypochloride and microzooplankton. We have recommended the use of sophorolipid to mitigate *Cochlodinium* red tide [1]. Nevertheless, with the exception of yellow clay, these methods have failed to be practically applicable because of high costs, negative side effects, or difficulty of use in the marine environment. It is well known that yellow clay is nontoxic, inexpensive, and easy to use, and has been applied to mitigate HABs in South Korea. To remove above 80% of HABs, dispersion concentration of more than 10 g L^{-1} yellow clay has been recommended. However, this high concentration of yellow clay has been a problem to produce anaerobic bottom environment and negative effects on other plankton and benthic algae. Compared with other surfactants, the sophorolipid showed a good algicidal effect and biodegradation efficiency, but low penetration efficiency into the water column.

To reduce the weak points of yellow clay and sophorolipid, we recommend to use the mixture of 1 g L^{-1} yellow clay and 5 mg L^{-1} sophorolipid. The sophorolipid-yellow clay mixture more efficiently mitigated the HAB (95% removal efficiency) than yellow clay alone (79%) and had a less adverse effect on bacterioplankton, heterotrophic protists and zooplankton than the yellow clay. These results indicate that sophorolipid-yellow clay mixture can mitigate HABs efficiently with environmental and economical effects.

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HABs early warning and mitigation with clay dispersal

To give timely assistance to fishermen and aquaculturists in advance of HAB, NFRDI has adopted a red-tide warning system. It consists of three steps: Red Tide Attention, Red Tide Alert and Warning Lifted. Attention and alert notices are issued when the density of fish-killing species, *C. polykrikoides*, exceeds 300 cells mL^{-1} and 1000 cells mL^{-1}, respectively. Once a red-tide warning is issued, NFRDI and local government recommend fisherman to stop feeding, and supply liquid oxygen and/or disperse yellow clay to minimize impact on resources. NFRDI also has a shellfish poisoning warning system based on the toxin levels of shellfish. When the PST level exceeds the safety limit, 800μg · kg^{-1} of shellfish meat, NFRDI issues a shellfish poisoning warning, and harvesting of shellfish is banned until the warning is lifted.

Mariculture for finfish and shellfish is widespread in coastal Korea. Many pen cages, particularly for finfish culture, are concentrated mostly along the southern coast where there are many islands enabling aquaculture facilities protected from typhoons. However, HABs of *C. polykrikoikes* have occurred since the 1990s in both inshore and offshore waters, and resulted in severe fisheries damage in the area. Yellow clay composed of montmorillonite has been applied since 1996 to minimize damage by *C. polykrikoides* blooms. It is estimated that the application of the clay control method has reduced annual losses from 95 million US dollars in 1995 to 0.2-22.3 million US dollars in 1996-2007. Several devices such as clay dispensers, automatic HAB alarm systems and electrolytic water generators have been developed for fisherman and responsible agencies, to help in early warning for HABs and as tools for effective HAB mitigation. Clay dispensers crumble native yellow clay into a fine size which increases HAB removal rates, and disperse it automatically into HAB affected areas. These have been available to HAB control agencies since 1997. Automatic HAB alarm system equipped with chlorophyll and turbidity sensors has been developed and made available to land-based finfish culture farms since 1999, and provide warning sound or signals when red tide is introduced into aquaculture sites. Another special device, the Electrolytic Clay Dispenser (ECD), an electrolytic water generator and clay dispenser combined, has been developed and deployed to local government since 2001 in Korea. The device is designed to minimize the amount of clay treatment applied, to reduce any ecological impact, particularly on the benthos by accumulation of clay on the sea bed, and to enhance HAB removal efficiency compared to earlier designs of clay dispenser. NFRDI has now started to monitor environmental changes including impacts on areas where yellow clay was frequently introduced during *C. polykrikoides* blooms since 1998. The impact on the fauna, particularly on benthos, is thought to be negligible based on present results, but further long term studies is needed.

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The Eco-STAR project to develop technology for aquatic ecosystem restoration and management

As a part of the Eco-STAR Project (part of Core Environmental Technique Development Project for Next Generation of the Korean Ministry of Environment), the Aquatic Ecosystem Restoration Project group (AERP) was established in 2007 to develop eco-friendly environmental techniques, to be promoted as a competitive project in the 21st century (Fig. 8). A sum of 84.1 billion Korean won (Government 58.5 billion + private 25.6 billion) is earmarked for the Eco-STAR budget, to develop technology for aquatic ecosystem restoration and management over 6.5 years (December 2007 to May 2014); the group has a total of 684 researchers from 75 institutes.

To restore and preserve the health of damaged aquatic ecosystems, AERP develops practical technologies in four areas, i.e., i) to restore and manage aquatic ecosystems; ii) to alleviate stagnation and to purify water quality; iii) to develop and to manage riparian margins, and iv) to evaluate aquatic ecosystem health, on the principle of "mutual survival of humanity and nature." The project also emphasizes the significance of the societal and economic value of aquatic ecosystem restoration, and education and public relations.

There are twelve sub-projects. One of these, the "Development of Nature-friendly Technology for Water Quality Purification", deals mainly with efficient control of harmful algal blooms (HABs) in freshwaters, by evaluating the performance of HAB control techniques using algicidal bacteria and algicidal substance(s) derived from them. Thus, the project involved i) isolation of various algicidal bacteria active against problem species in South Korea; ii) identification of algicidal substances extracted from these bacteria, and iii) improvement of existing algicides into useful chemical agents (hence termed 'MEDI-TIDE') possessing stronger algicidal activity, more species-specific activity and lower toxicity. At present, iv) the possible use of MEDI-TIDE for the efficient termination of natural blooms of problem species with minimal adverse effects on freshwater systems (reservoirs and rivers) is being assessed under field conditions. Finally, further study of the optimization of mass-production of MEDI-TIDE is planned.

Fig. 8. The logo of Aquatic Ecosystem Restoration Project Group signifies the unification of humans and nature. Expressing the shape of a human's body with raised hands in calligraphy, it describes free human image and the wave lines in blue and green express water and forest, respectively. The harmony of two colors expresses the image of human and nature that advance toward the future together. As warm colors, those used for the shape of human mean passion, energy and challenging spirit. Source: Myung soo Han, Han Yang University, hanms@hanyang.ac.kr

Many blooms represent a serious threat to the use and sustainability of our freshwater resources. The commonest problems (toxicity, food web altering, hypoxia generating), are due to *Microcystis, Anabaena, Stephanodiscus,* and *Peridinium*, and affect drinking water supplies from both health and economic perspectives. These are found annually in most lakes, reservoirs, and rivers, including the Pal'tang (37 °N and 127 °E), Juam (34-35 °N and 127 °E), and Daechung Reservoirs (36 °N and 127 °E) and Nakdong River (35-37 °N and 124-131 °E) of South Korea. Thus, if the project is successfully completed as planned, MEDI-TIDE may be a simple and environmentally friendly solution for the provision of safe potable water, and also effectively prevent blooms and curtail excess algal and cyanobacterial growth.

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Use of molecular probes to understand migration, dispersal and population dynamics of harmful algal bloom species

The first outbreak of *Cochlodinium polykrikoides* was observed in the Nak-Dong estuary near Busan, Korea in 1982. Since then, *Cochlodinium* blooms have occurred in Tongyeong, Geoje, Namhae and Yeosu in southern Korean coastal waters [1, 2]. Finally, it became widespread from the West to the East Sea via southern Korean coastal waters [1, 3]. The frequency and intensity of *Cochlodinium* blooms has increased over time [4] and cell density exceeded 48,000 cells ml^{-1} in 2003. *C. polykrikoides*-related fish kills have gradually increased, with huge economic losses, over 100 million US dollars in Korean coastal waters (NFRDI report) (Fig. 9).

Nevertheless, we still do not fully understand the migration, dispersal and bloom mechanisms of *C. polykrikoides*, about which there are many questions (Fig. 10). Previous research reported that *C. polykrikoides*'s bloom begins each year near Narodo in southwest coastal waters of Korea. But, it is unclear whether *C. polykrikoides* blooms were advected by the Tsushima and Yellow Sea warm currents, branches of the Kuroshio Current, or germinated from seed populations near Narodo. Moreover, *C. polykrikoides* blooms also last for longer periods than other red tides [4]. Some studies suggested that assemblages of *Cochlodinium* (Fig. 11) may be composed of two or more strains or species. Recently, a *C. polykrikoides* population was classified into East Asian (Korean/Japanese), Philippines and American/Malaysian ribotypes by 28S ribosomal DNA [5]. However, it is impossible to identify each *C. polykrikoides* ribotype microscopically due to extremely similar morphologies. Nothing is known about the geographical distribution of these *C. polykrikoides* ribotypes in Korean coastal waters.

Molecular techniques such as FISH (Fluorescence In situ Hybridization), microsatellite markers and quantitative real-time PCR assays are necessary to detect and quantify each *C. polykrikoides* ribotype. Surveys were performed weekly or bi-weekly from June to November in southern coastal waters of Korea for 3 years (2009-2011). Physical (temperature, pH, DO and salinity), chemical (nutrients and chlorophyll a) and biological factors (phytoplankton, bacteria, HNF, ciliate and micro-zooplankton etc.) were investigated. We selected 35 sampling sites from July to August in 2008 to determine the geographical distribution of each *C. polykrikoides* ribotype. In order to understand the migration and dispersion of *C. polykrikoides*, sampling was extended into the East China Sea, Jeju and Tsushima Island. Then distribution of *C. polykrikoides* ribotypes was analyzed with quantitative real-time PCR assay.

As results of this project, we discovered that Philippines and East Asian ribotypes are widely co-distributed all around Korean coastal waters. The Philippines ribotype was detected even in the East China Sea and around Jeju Island. This implies that the Philippines ribotype may flow into Korean coastal waters via the Yellow Sea warm current. In 2009, we found a mixed bloom of *C. polykrikoides* in which Philippines (summer) and East Asian (autumn) ribotypes showed different seasonal fluctuation in southern Korean coastal waters. These results suggest that the long term persistence of *C. polykrikoides* blooms may be caused by a succession of different ribotypes in the same area.

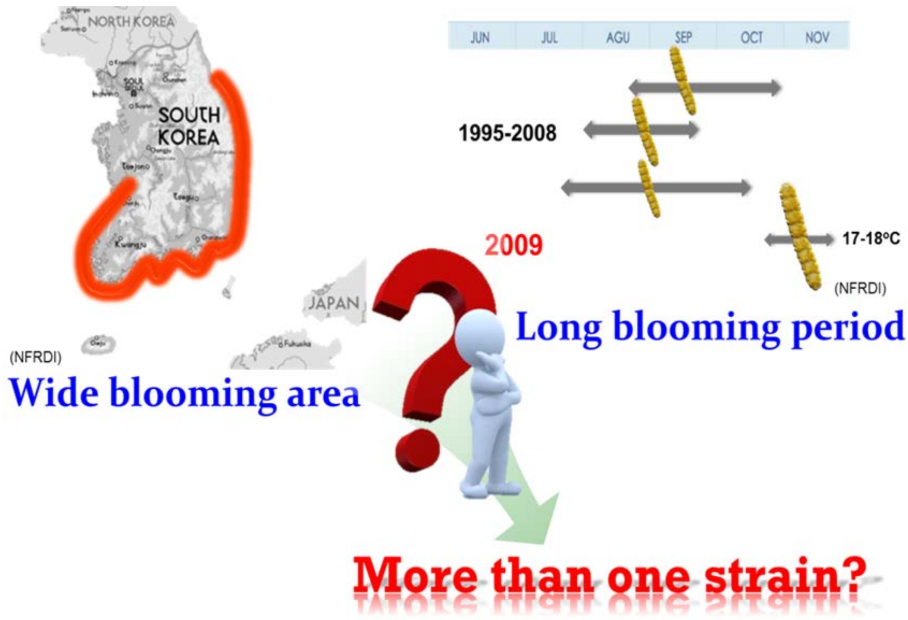

Fig. 9. Huge long-term bloom of Cochlodinium polykrikoides *in Korean coastal waters. Source: Myung soo Han, Han Yang University, hanms@hanyang.ac.kr .*

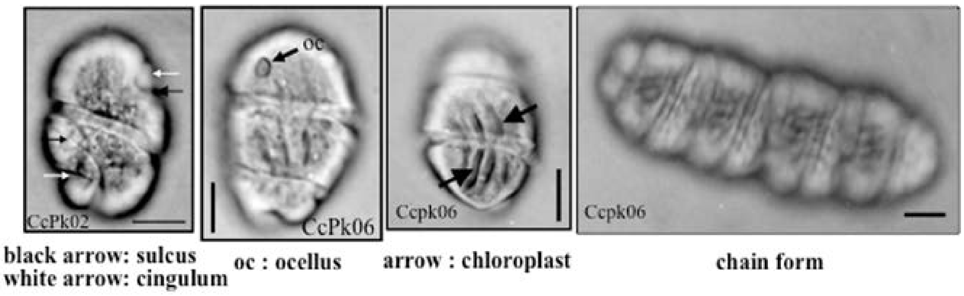

Fig. 10. Micrographs of the red-tide chain-forming dinoflagellate Cochlodinium polykrikoides *(scale bar 10 µm). Source: Prof. MS Han.*

Fig. 11. Harmful algal blooms scum visible in Daechung, South Korea (from http://www.newsis.com/, ©Newsis, 2010).

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Karenia blooms in Gulf of Mexico

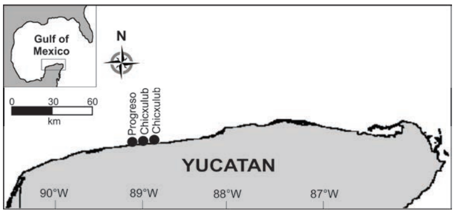

Fig. 1. Sampling sites at the northern coast of the Yucatan Peninsula in August 2011.

Table 1. Abundance of Karenia *species in the Progreso-Chicxulub area, SE Gulf of Mexico (cells L^{-1}).*

Sampling site	*K. brevis*	*K.* cf. *papilionacea*	*K. papilionacea*	Total
Progreso	15808	20072	7280	43160
Chicxulub 1	22500	45443	9557	77500
Chicxulub 2	7480	4307	170820	182607

During the last two years, blooms caused by *Karenia* species in the southern Gulf of Mexico have increased in frequency. In the central part of the State of Veracruz, a mixed bloom of *K. brevis* and *Karenia* "Mexican hat" sp. was reported in 2010 [1].

Karenia species appeared in high abundances in the Progreso-Chicxulub area (21°21.421'-21°22.466'N, 89°00.832'-89°07.333'W; Fig. 1; Table 1) at three coastal sites out of 16 visited monthly since 2002. The ranges of the physical-chemical parameters were as follows: temperature 26.7-27.5°C, salinity 36.20-36.32, nitrates 6.75-6.97 µM, nitrites 0.12-0.22 µM, ammonium 0.99-1.80 µM, urea 3.21-4.37 µM, phosphates 0.052 µM (the only measurement), silicates 2.24-4.16 µM, and chlorophyll "a" 0.38-0.46 mg m^{-3}.

We were able to identify two taxa at species level in water-bottle samples taken in the surface layer, using an inverted Olympus CKX41 microscope in a Sedgwick-Rafter chamber. *Karenia brevis* (Fig. 2 and 3) and *K.* cf. *papilionacea* (Fig. 4 and 5) were rather abundant at two sites, and *K. papilionacea* dominated at the easternmost locality (Fig. 6 and 7). However, most of cells remained unidentifiable under the light microscope due to distortion in cell shape after fixation, and we designated them conventionally as *K.* cf. *brevis and K.* cf. *papilionacea* (Fig. 4 and 5). In all examined cells of *Karenia* species, the nucleus was located in the left part of the hypotheca. In acid Lugol-fixed samples, the details of the sulcus, the cingulum and the apical grove were not seen. The length (L) and the width of cell (W) and the W/L ratio were measured for 199 cells. The morphometrical characteristics for *K. brevis* (including *K.* cf. *brevis*) were as follows: L=17.5-29.5 µm (19.77±1.84), W=19-31.5 µm (24.70±3.08), W/L=0.85-1.53 (1.25±0.12), n=158; for *K. papilionacea* (including *K.* cf. *papilionacea*) L=18-25 µm (21.45±1.42), W=29-37.5 µm (31.49±2.29), W/L=1.33-1.7 (1.47±0.10), n=41.

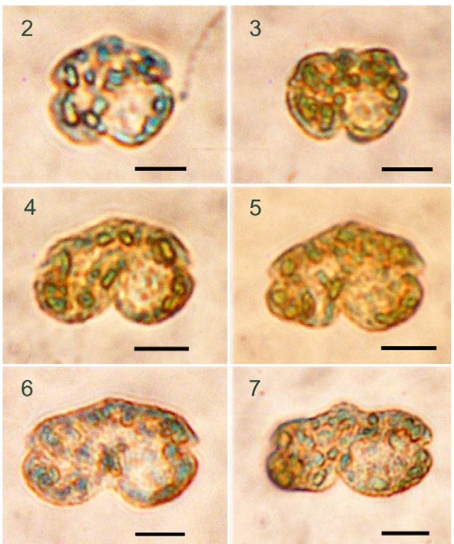

Fig. 2-7. Karenia *species from northern Yucatan: 2-3* K. brevis; *4-5* K. *cf.* papilionacea; *6-7* K. papilionacea. *Scale bar: 10 µm.*

To our knowledge, this is the first *Karenia* bloom ever registered along the northern coast of Yucatan Peninsula. Both species mentioned above are known as either producers of brevetoxins or brevetoxin-like compounds [2]. Judging from the recently reported events to the west of the study area, we can expect various *Karenia* species in the SE Gulf of Mexico. According to unpublished data of the state laboratories of public health, *K. brevis* and *K. papilionacea* were also observed on 15 September 2011 in coastal waters of the northern part of the State of Campeche, SW of our study area [3], and on September 22-24 offshore [4]; in August-September 2011, *K. brevis, K. mikimotoi* and *K. selliformis* blooms in the coastal waters of the State of Tabasco, further to the west of the State of Campeche [5]. All the species mentioned above have been found in the Gulf of Mexico [2], and we speculate that the latter two and *K. papilionacea* will be found in the SW Gulf of Mexico in the very near future because of physical transport mechanisms. In the northern Gulf, *K. brevis* has been reported from Texas to Florida, and even up the east coast [6]. *Karenia papilionacea* occurs off Texas, Louisiana, and Florida [7, 2, 8], and based on the inferred transport of the 1996 bloom from Florida to Louisiana this species should occur off Alabama and Mississippi.

Acknowledgements

Financial support to the FOMIX CONACYT-Yucatan project to JAHS is much appreciated. Thanks to Ileana Osorio-Moreno for chemical analyses, Ana C. Aguilar-Trujillo for the map, Natalia Okolodkova for help with the photomicrographs, and to Fausto Tafoya del Ángel and Olga Elena Piña-Gutiérrez for the Campeche and Tabasco data.

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First report of Dinophysis acuminata bloom in Basque waters, Northern Spain

Phytoplankton has been monitored since 2000 in the Nervión River estuary in the Southern Bay of Biscay (Northern Spain), since 2002 in the coastal waters and estuaries of the Basque Country, and since 2005 along the Cantabria coast. Since then, several toxin-producing harmful species has been identified, including several species of *Pseudo-nitzschia*, dinoflagellates *Dinophysis, Karlodinium, Karenia, Ostreopsis* and *Prorocentrum*, and flagellated forms such as *Prymnesium* spp. and *Heterosigma akashiwo* [1, 2, 3]. The genus *Dinophysis* is one of the most frequently found in the area, but generally appears in very low concentrations (< 100 cells L^{-1}). However, during the spring survey of 2011, high concentrations of *Dinophysis acuminata* complex were observed at two coastal stations, one (L-N20) 2 km from the coast and 4.5 km from the mouth of Nervión River, the other (L-B10) 0.7 km from the coast and 2.7 km from the mouth of the Butron River (Fig. 1). Cells in surface samples were counted by the Utermöhl method. Cell concentrations reached 5×10^3 cells L^{-1} at st. L-N20 and 1.8×10^4 cells L^{-1} at st. L-B10. Other dominant taxa in the samples were the coccolithophore *Emiliania huxleyi*, with densities around 7×10^5 cells L^{-1}, and the haptophyte *Chrysochromulina*, the prasinophyte *Pachysphaera* and the diatom *Pseudo*-nitzschia, all of them with concentrations around 5×10^4 cells L^{-1}.

The identification of the genus *Dinophysis* is largely based on the cell contour, the size and shape of the large hypothecal plates, and the shape of the left sulcal lists and their ribs [4]. Taking into account the average measurements of the cells observed (maximum length: 45 µm; dorso-ventral depth: 30 µm) (Figs. 2 and 3), and based on Raho et al. [4], the specimens were identified as *Dinophysis acuminata*, but genetic methods should be applied for a more certain identification.

Dinophysis can produce diarrheic shellfish poisoning (DSP), a major cause of economic losses to shellfish aquaculture, which makes this genus one of the most studied harmful taxa [5]. It is well known that *Dinophysis* species prey on *Mesodinium rubrum*, but on this occasion the ciliate was not observed in the samples; but the highest densities of *Dinophysis* and *Mesodinium rubrum* do not necessarily coincide, and *Dinophysis* peaks may be as much as a month later than the highest densities of *Mesodinium rubrum* [6].

Dinophysis is also reported in very low concentrations in other areas, with values of the order of 10-40 cells L^{-1} [5]. However, with increased the spatial and temporal resolution of sampling, it has been found that the genus can reach bloom proportions more frequently than previously observed. Blooms can take place at different depths, and be concentrated by currents or by vertical migration and subsequent growth. Under favorable conditions of light and prey availability, *Dinophysis* species can attain very high growth rates [5]. The difficulty in finding blooms of *Dinophysis* despite its recurrent presence in coastal waters can to some extent be explained by its aggregation in thin layers of 10 cm to 3 m, which can extend horizontally for many kilometers [6].

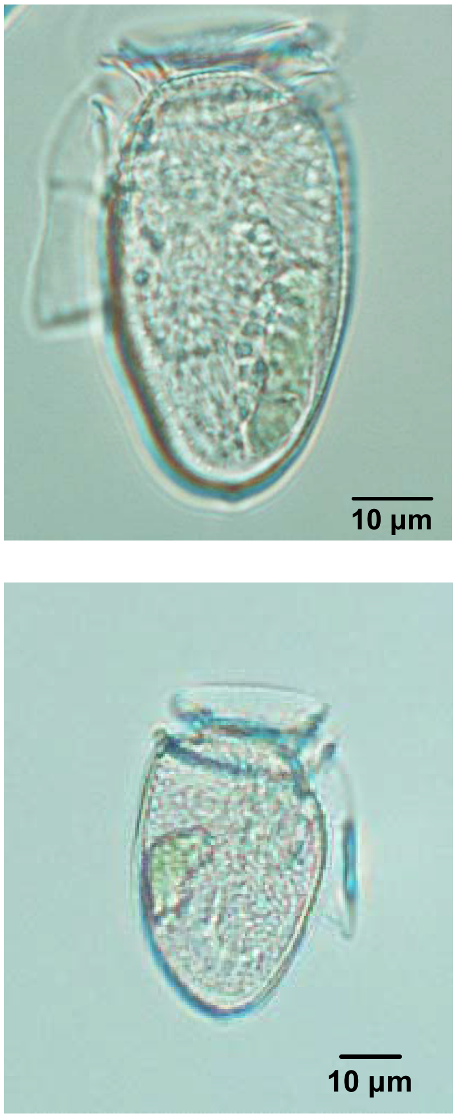

Fig. 2. Micrographs of Dinophysis acuminata *complex*

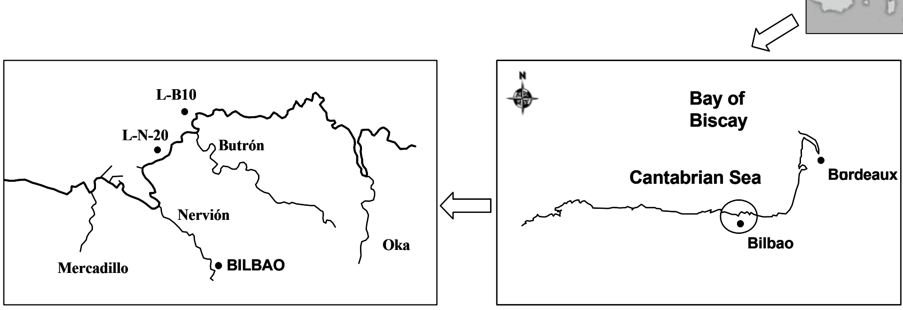

Fig. 1. Location of the two stations

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Dinoflagellate Bloom Coincides with Marine Invertebrate Mortalities in Northern California

Harmful Algal Bloom (HAB) events that involve non-traditional HAB species are poorly understood but can nonetheless cause extensive marine life mortalities. Documenting these events will be important for increasing our understanding of the ecological impacts of HABs on marine ecosystems. One such algal bloom coinciding with a mortality event impacting marine invertebrates was recently documented in Sonoma County, north of San Francisco, California (USA) in August 2011 (Fig. 1). Reports of this bloom and the marine invertebrate mortalities extended 80km along the nearshore from Bodega Bay: (Lat. 38.33N – Long. 123.02W) north to Anchor Bay, California (Lat. 38.80N – Long. 123.58W). Ocean conditions were calm in the weeks prior to the die-off, and there was an extraordinary display of night-time bioluminescence which persisted through the initial observations of dead invertebrates. Daily samples taken before, during and after the mortality event show the phytoplankton community was dominated by a dinoflagellate in the *Gonyaulax spinifera* (R. Horner pers. comm.) species complex (Fig. 2). SEM images of the bloom samples revealed that two morphotypes of *G. spinifera* were present.

To our knowledge, this is the first confirmed report of an algal bloom which caused invertebrate mortalities in a suite of economically and ecologically important species in northern California. Previously observed die-offs in this region were small in scale and appeared to be associated with low dissolved oxygen conditions following algal blooms or the degradation of drift macroalgae accumulations in protected coves. There have been blooms of the dinoflagellate *Cochlodinium* resulting in invertebrate mortalities along the central and southern Califonia coast, including the loss of over 1 million seed oysters in Morro Bay. The cause of this southern California mortality appeared to be associated with a toxin or something other than oxygen depletion.

The invertebrates that died during the northern California bloom included herbivores and carnivores from multiple phyla including mollusks, echinoderms and crustaceans, but fish deaths were not observed. After dead shellfish and sea stars washed ashore (Fig. 3), quantitative subtidal field surveys in the kelp beds in Sonoma County were conducted. Surveys revealed that at four sites an average of 25% of red abalone, *Haliotis rufescens*, a recreationally fished species and red sea urchins, *Strongylocentrotus franciscanus*, a commercially fished species were dead or dying. Non-fished species were also impacted including gumboot chiton, *Cryptochiton stelleri*, other mollusks, purple sea urchins, *S. purpuratus*, and several species of sea stars including *Pisaster ochraceous*. The spatial patterns of mortality included both coves with reduced water flow and the potential for localized oxygen depletion and exposed headlands where oxygen depletion is highly unlikely. Invertebrates in shallow water (<10m) suffered greater mortality than those in deeper water. Invertebrates within the Bodega Marine Lab (BML) located within the affected region also died during this event, with sea stars exhibiting bizarre arm bending behavior prior to dying, but fish were not affected. Oxygen levels measured in one tank at the lab were normal (97% of full saturation) during the event. Some invertebrates appeared unaffected by the event such as the bat star, *Asterina miniata,* which was observed actively scavenging dead abalone and sea urchins in the field. Using these data, the Fish and Game Commission in California voted in September, 2011 to close the recreational abalone fishery in Sonoma County for the remainder of the 2011 season.

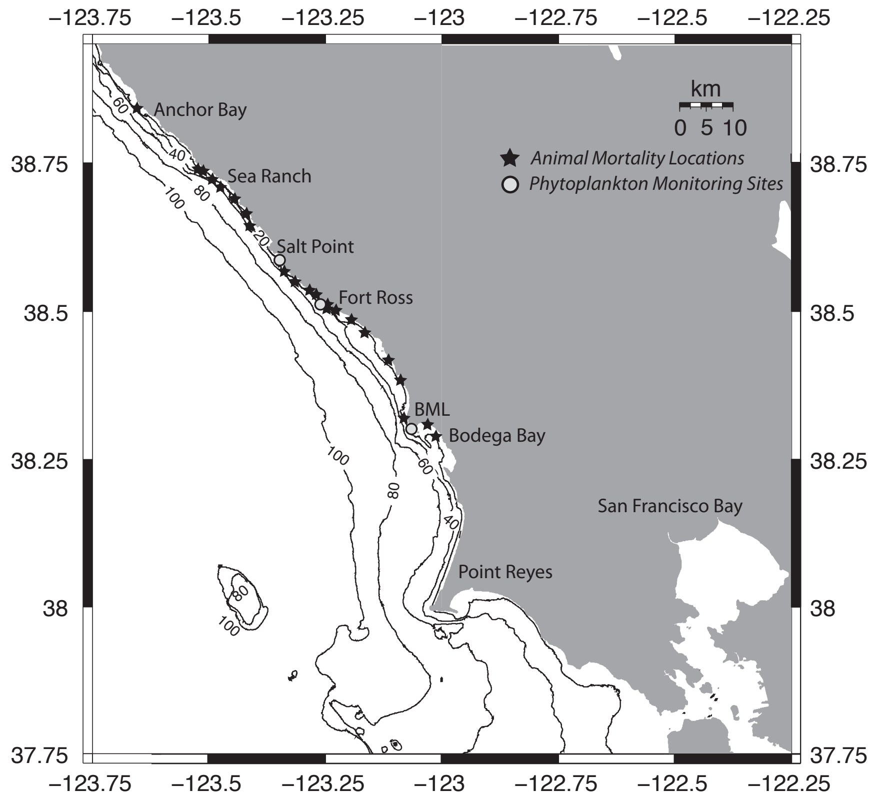

Fig. 1. Map of region in northern California impacted by the harmful algal bloom.

Mussels, *Mytilus californianus,* which bioconcentrate phytoplankton toxins, were collected during the bloom and tested by the California Department of Public Health for Paralytic Shellfish Poisoning (PSP) using the Jellett Rapid Testing Systems lateral flow immunoassay and Domoic Acid toxin using the standard High Performance Liquid Chromatography (HPLC) method [1]. Test results for these toxins were below the minimum detection limits. Two independent labs at the University of California, Santa Cruz (R. Kudela UCSC) and Fish and Game Laboratory at OSPR (D. Crane) detected trace levels of Yessotoxin (YTX) using HPLC-mass spectrometry (UCSC) and tandem mass

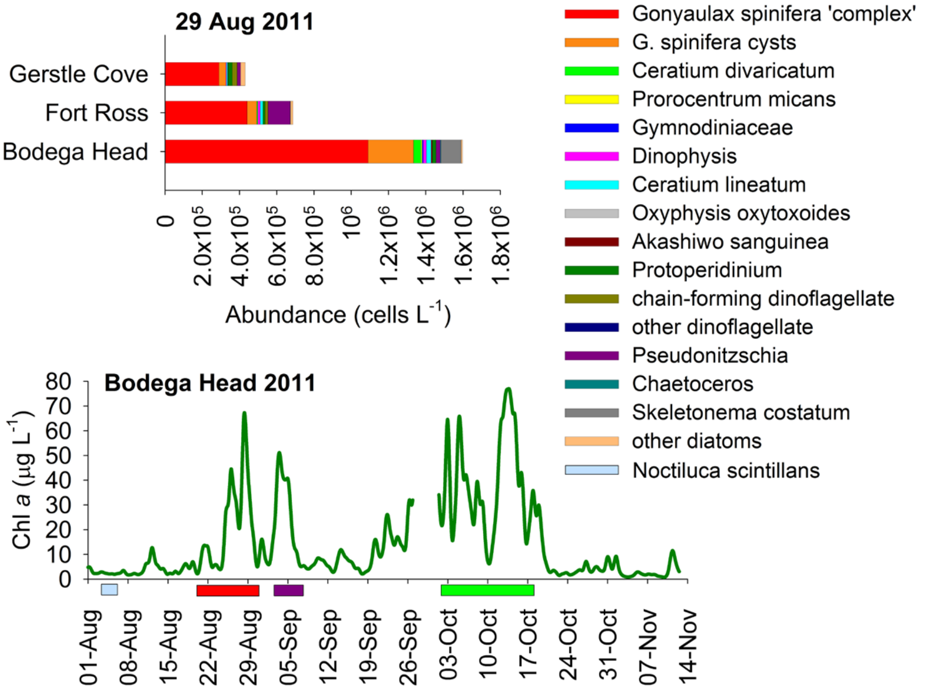

Fig. 2. Chlorophyll a time series showing dominant taxa during visible bloom events at Bodega Head and phytoplankton species abundances from 3 locations in Sonoma County (noted in Fig. 1 with open symbols) where invertebrate die-offs occurred.

spectrometry (OSPR) in both mussels and abalone gut tissue. *Gonyaulax spinifera* is known to produce YTX [2]. The US Food and Drug Administration lab in Dauphin Island, Alabama detected trace levels of YTX, dinophysistoxins and okadaic acid, as well as pectenotoxins, and spirolides. UCSC independently confirmed that other common toxins including domoic acid, okadaic acid, and microcystins were below detection. Histology of the tissues including the gastrointestinal tract, kidney, nervous tissue, gonad, muscle and gills from abalone that died at BML as well as red abalone collected from Fort Ross two weeks after the peak event showed no consistent pathologic changes.

To our knowledge, this is only be the second time YTXs have been detected in California [3]. YTX is a class of disulfate polyether (lipophilic) toxins with at least 90 different analogues identified from phytoplankton and shellfish although the structures of just a few are known [4]. The oral potency of YTX is thought to be exceedingly low, however it is toxic when injected into mice [5]. Abalone and sea urchins are herbivores which feed primarily on drift macroalgae while the sea stars affected here are carnivores that feed on mussels and other invertebrates. The trophic transfer of a toxin from the phytoplankton to non-filter feeding herbivores is unclear as is the oral toxicity of the YTX. Incidental ingestion of the dinoflagellate and/or its cysts on macroalgae is one possible route of exposure as observed with herbivorous manatees dying after consuming sea grass coated with dinoflagellate [6]. Work currently underway is examining the genetics of the *Gonyaulax spinifera* species complex, the mechanism of toxin transfer, as well as any change in the genetic structure of the abalone populations in the region as a consequence of the event. Since *Gonyaulax* forms cysts, the possibility cannot be ruled out that a similar bloom event may occur in the same geographic region in the future.

Fig. 3. Dead red abalone and gumboot chitons wash ashore at Fort Ross Cove, Sonoma County, California August 2011. Photo Credit: Nate Buck.

Acknowledgements

We thank Quay Dortch and CSCOR HAB Event Response Program (NOAA) for expertise and support as well as the Central and Northern California Ocean Observing System (CeNCOOS). We also thank California Sea Grant and the California Ocean Science Trust for hosting a workshop related to HAB event response. This publication is a contribution of the Bodega Marine Laboratory, University of California, Davis.

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A Recurring Bloom of Toxic Marine Cyanobacteria above the Arctic Circle

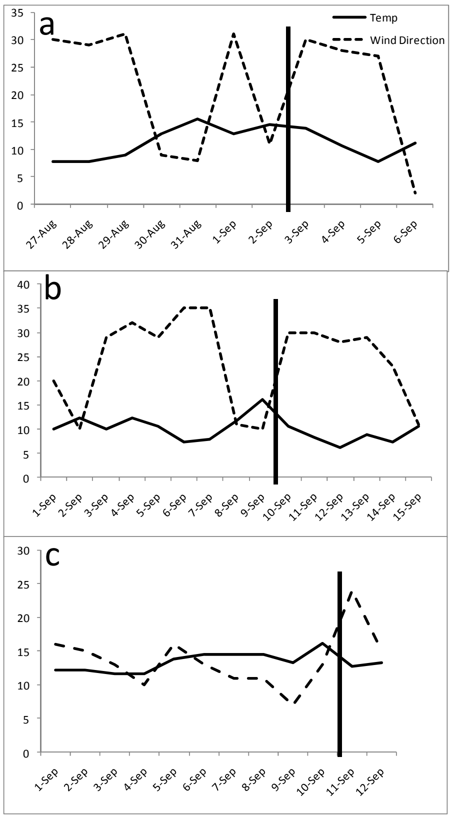

Fig. 2. Air temperature (solid line) and Wind Direction (dotted line) data collected from Wein Memorial Airport over time in 2008 (A), 2009 (B) and 2010 (C). Temperatures are given in degrees Celsius and wind direction is described in tens of degrees clockwise from true north. A value of 36 indicates North, 27 indicates West, 18 indicates South, 9 indicates East and a value of 0 is calm. Note the differences in the x and y axes between the graphs. The vertical solid lines represent the approximate initiation of the bloom.

During September of 2008, the waters of Kotzebue Sound, Alaska, turned a fluorescent green color. Given that Kotzebue Sound is above the Arctic Circle, local authorities initially suspected chemical contamination and not a biological origin. Samples were collected and sent to the Alaska Department of Environmental Conservation by the Alaska Department of Fish and Game Kotzebue Office but only tested for the presence of petroleum and petroleum byproducts. A year later (September 10, 2009), another bloom with a "grass seed" appearance occurred in Kotzebue Sound. This bloom was identified by light microscopy as the cyanobacteria, *Aphanizomenon flos-aquae* [1]. This was followed on September 10, 2010, by another bloom that was identified as a mixture of *Aphanizomenon* sp. (3.1×10^5 filaments mL^{-1}), *Nodularia* sp. (5×10^4 filaments mL^{-1}) and *Anabaena* sp. (1×10^4 filaments mL^{-1}; Fig. 1). A cyanobacteria bloom also occurred August 26, 2011, but was very short in duration and was not sampled.

Observational data collected by a Kotzebue resident in a Weather Journal and meteorological data from a nearby weather station (National Weather Service weather station at the Kotzebue Wein Memorial Airport (http://weather.noaa.gov/weather/current/PAOT.html) suggested that in 2008-2010, a combination of ~15.5 °C air temperatures, South to East winds and a rain event followed by clear skies can combine during an overall warm, dry summer to create conditions favorable for the initiation of these cyanobacteria blooms (Fig. 2).

Toxin analyses [2, 3] showed that the blooms were positive for microcystins and/or nodularins in both 2009 and 2010 (Table 1) using the bioactivity-based protein phosphatase inhibition assay. However these two years showed very different toxin profiles when analyzed using a liquid chromatography assay coupled with mass spectrometry. In 2009, only microcystin-LR was detected, though the small sample size (50 mL) would have prevented detection of minor congeners accounting for less than 10% of the total. In contrast, 2010 samples contained no detectable microcystins but consisted solely of the arginine isomer of nodularin (Nod-R, *m/z* MH+=825). Other common cyanobacteria toxins (anatoxin-a, cylindrospermopsin and the PSP toxins) were not observed in these samples and were either not present or below our detection limits (Table 1).

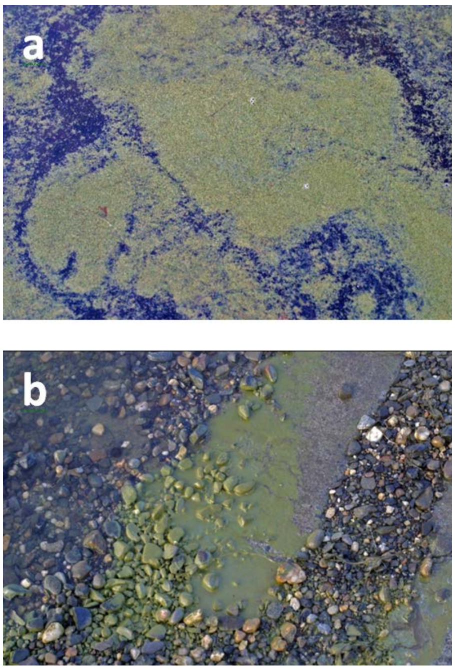

Fig. 1. Photographs of the Aphanizomenon flos-aquae *bloom taken in 2010 A. on the surface of Kotzebue Sound and B. on the shoreline.*

Aphanizomenon species are cosmopolitan and production of the toxins anatoxin-a, cylindrospermospin, and the paralytic shellfish toxins (saxitoxin and its derivatives) has been reported for a number of freshwater members of this genus [4]. Marine species in the order Nostacales produce both microcystins (*Anabaena* species, [5]) and nodularins (*Nodularia spumigena*, [6]), but neither microcystin, as observed in 2009, nor nodularin, as observed in 2010, has been associated with *Aphanizomenon* species in fresh or brackish waters. High values of nodularin production similar to that observed in 2010 have been reported for *Nodularia spumigena* blooms in the Baltic Sea [7].

Cyanobacteria samples collected from the bloom in 2009 and 2010 had their ITS rRNA region extracted, amplified and sequenced [8, 9, 10]. These sequences were compared to others in GenBank. The BLASTn results outlined in Tables 2 and 3 show that the molecular data support the morphological identification of *Aphanizomenon flos-aquae* in the 2009 and 2010 samples. In a 268 base pair region, twenty-two clones spanning the two years had a BLASTn result of 98% and 97% with characterized *A. flos-aquae* strain 83 (Denmark) and strain 326 (Finland), respectively. It is quite possible that generation of more clones would have unveiled other species present. Likewise, using this molecular strategy we uncovered a species (*A. lemmermannii*) belonging to one of the other genera in the 2010 sample identified morphologically (*Anabaena*). There are several *A. lemmermannii* ITS sequences available on GenBank (in addition to the UK sequences present in our BLASTn results) which were derived from characterized cultures [11], but did not reveal them-

Table 1. Concentration and detection limit for different cyanobacteria toxins in a sample collected in September 2009 and 2010 from Kotzebue Sound. For comparison purposes, microcystins and nodularins as determined by the protein phosphatase inhibition assay (PPIA) and by liquid chromatography mass spectrometry (LCMS) are expressed in terms of microcystin LR equivalents.

2009

Toxin	Method	Detection limit	Results
Total Microcystins/Nodularins	PPIA	1.2 µg L^{-1}	4.7 µg L^{-1}
Micro./Nod. congeners	LCMS	1.3 µg L^{-1}	3.5 µg L^{-1}
Anatoxin-a	LCMS	1.0 µg L^{-1}	Below detection
Cylindrospermopsin	LCMS	0.6 µg L^{-1}	Below detection
PSP Toxins	ELISA	13.6 µg L^{-1}	Below detection

2010

Toxin	Method	Detection limit	Results
Total Microcystins/Nodularins	PPIA	1.2 µg L^{-1}	790 µg L^{-1}
Micro./Nod. congeners	LCMS	7.0 µg L^{-1}	600 µg L^{-1}*
Anatoxin-a	LCMS	0.2 µg L^{-1}	Below detection
Cylindrospermopsin	LCMS	0.2 µg L^{-1}	Below detection
PSP Toxins	ELISA	13.6 µg L^{-1}	Below detection

*equivalent to 495 µg nodularin-R L^{-1}

Table 2. Top BLASTn results for the consensus sequence from ITS clones from the 2009 (n=12) and 2010 Kotzebue samples (n=10). Six additional clones from the 2009 sample and five from the 2010 sample shared >99% similarity but are not included in the groupings because they contained one polymorphism (one additional from 2010 showed 96 - 98% similarity) with the BLASTn results (data not shown). The query sequence was 362 nucleotides. Only BLASTn results with sequence similarity of 96% or greater are included.

Clone (*n*)	BLASTn result [9]	Sequence designation	location	Ref	GenBank Accession number	Nucleotide overlap	% similarity
2009a (12) JQ433686 2010a (10) JQ433693	Uncultured cyanobacterium	DGGE band #26	Netherlands	14	AY827756	362	99
	Uncultured cyanobacterium	DGGE band #14	Netherlands	14	AY827744	321	99
	Aphanizomenon flos-aquae	DGGE band HV25b	Baltic Sea	15	AF431745	268	99
	Aphanizomenon flos-aquae	DGGE band LJ38	Baltic Sea	15	AF431746	268	99
	Uncultured cyanobacterium	DGGE band #44 and #52	Netherlands	14	AY827782	362	98
	Uncultured cyanobacterium	DGGE band e19	Netherlands	16	AJ619651	322	98
	Uncultured cyanobacterium	DGGE band #9	Netherlands	14	AY827739	322	98
	Uncultured cyanobacterium	DGGE band #19	Netherlands	14	AY827749	319	98
	Aphanizomenon flos-aquae	DGGE band PB98	Baltic Sea	15	AF431747	268	98
	Aphanizomenon flos-aquae var. *klebahnii*	strain 83	Denmark	11	AJ293201	268	98
	Aphanizomenon flos-aquae	DGGE band LJ31	Baltic Sea	15	AF431754	268	97
	Aphanizomenon flos-aquae	strain 326	Finland	11	AJ293200	268	97
	Aphanizomenon flos-aquae	DGGE band BSW9	Baltic Sea	15	AF431755	237	97
	Uncultured cyanobacterium	DGGE band #15	Netherlands	14	AY827745	335	96
	Uncultured cyanobacterium	DGGE band #18	Netherlands	14	AY827748	333	96

Table 3. BLASTn results for six unique ITS clones derived from the 2010 Kotzebue sample. Only BLASTn results with sequence similarity of 96% or greater are included.

Clone (*n*; length)	BLASTn result [9]	Sequence designation	location	Ref	GenBank Accession number	Nucleotide overlap	% similarity
2010a (2; 366) JQ433700	*Anabaena lemmermannii*	BC Ana 0004	United Kingdom[1]	Unpubl.	AY886938	273	99
	Anabaena lemmermannii	BC Ana 0018	United Kingdom[1]	Unpubl.	AY886942	273	98
	Anabaena lemmermannii	BC Ana 0017	United Kingdom[1]	Unpubl.	AY886941	273	98
2010b (1; 362) JQ433701	Uncultured cyanobacterium	DGGE band #9	Netherlands	14	AY827739	322	97
	Uncultured cyanobacterium	DGGE band #19	Netherlands	14	AY827749	319	97
	Anabaena lemmermannii	BC Ana 0004	United Kingdom[1]	Unpubl.	AY886938	257	97
	Uncultured cyanobacterium	DGGE band #15	Netherlands	14	AY827745	335	96
	Anabaena lemmermannii	BC Ana 0018	United Kingdom[1]	Unpubl.	AY886942	273	96
	Anabaena lemmermannii	BC Ana 0017	United Kingdom[1]	Unpubl.	AY886941	273	96
2010c (1; 366) JQ433702	Uncultured cyanobacterium	DGGE band #14	Netherlands	14	AY827744	305	97
	Anabaena lemmermannii	BC Ana 0017	United Kingdom[1]	Unpubl.	AY886941	273	96
2010c (1; 366) JQ433702	Uncultured cyanobacterium	DGGE band #14	Netherlands	14	AY827744	305	97
2010d (1; 367) JQ433703	Uncultured cyanobacterium	DGGE band #14	Netherlands	14	AY827744	306	96
	Uncultured cyanobacterium	DGGE band #19	Netherlands	14	AY827749	306	96
	Uncultured cyanobacterium	DGGE band #9	Netherlands	14	AY827739	306	96
	Anabaena aphanizomenoides	DGGE band K47-m3	Netherlands	14	AY827806	287	96
	Aphanizomenon flos-aquae	DGGE band LJ38	Baltic Sea	15	AF431746	257	96
	Aphanizomenon flos-aquae	DGGE band LJ31	Baltic Sea	15	AF431754	256	96
	Aphanizomenon flos-aquae	DGGE band BSW9	Baltic Sea	15	AF431755	238	96
2010e (1; 362) JQ433704	*Anabaena lemmermannii*	BC Ana 0004	United Kingdom[1]	Unpubl.	AY886938	257	97
	Uncultured cyanobacterium	DGGE band #9	Netherlands	14	AY827739	322	96
	Uncultured cyanobacterium	DGGE band #19	Netherlands	14	AY827749	319	96
	Anabaena lemmermannii	BC Ana 0018	United Kingdom[1]	Unpubl.	AY886942	257	96
	Anabaena lemmermannii	BC Ana 0017	United Kingdom[1]	Unpubl.	AY886941	257	96

[1]Cotswold Water Park

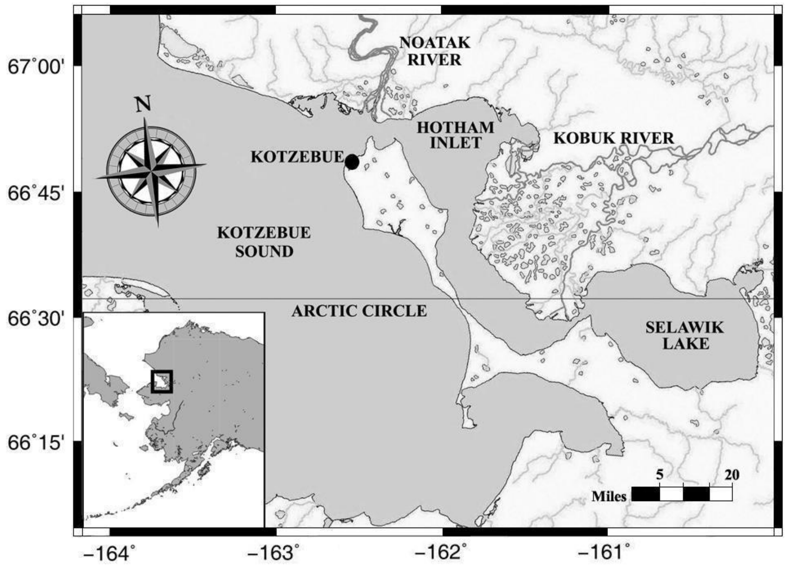

Fig 3 Map of Kotzebue Sound

selves as close matches. Upon further alignments between these sequences, the UK *A. lemmermannii* and our clones 2010a, 2010b and 2010e, it is apparent that these sequences are quite different (data not shown), calling into question the species designation of the sequences available on GenBank. The UK sequence data are unpublished, however the information on GenBank implies that these sequences were derived from isolates. As with many genera and species, more data are needed from characterized cultures/isolates to resolve these gaps in understanding.

Although ITS sequences for *Nodularia* (the third genus identified morphologically in the 2010 sample) are available on GenBank, this genus did not appear in any of our BLASTn analyses. It is quite possible that a more comprehensive clone library (i.e. a greater number of clones) would contain sequences from *Nodularia*. In addition, further analysis of these samples with species/genera-specific PCR would aid in further resolution of the cyanobacterial communities present.

Kotzebue Sound in northwestern Alaska is a partially enclosed embayment adjacent to the Chukchi Sea (Fig. 3). It is fed by three major rivers: the Noatak, Selawik and Kobuk. The Kobuk River is the largest of the three, draining an area of approximately 36,000 square miles and bordered by tundra with some evergreen forest. Salinity in the Sound is highly variable (3-28 during August-September; [12]) and dependent on river input, wind direction during the open water period, ice formation and ice melt during the ice-covered period.

The area is sparsely settled with the town of Kotzebue (3,100 residents) located on the Baldwin Peninsula on the shores of the Sound (Fig. 3). One additional small permanent settlement (Deering; <125 residents) and a few collections of seasonal campsites are also present along the Sound. Many of the residents are native Alaskans who harvest caribou, seal, fish and berries to meet their nutritional needs, with Kotzebue Sound providing 70% by weight of the annual harvest [13]. The Kotzebue Sound watershed has been used in this manner for at least the last few thousand years. Here we combine recent observations and modern molecular and analytical techniques to describe a recurring bloom of toxin-producing cyanobacteria in Kotzebue Sound. The toxicity of this bloom and the reliance of local residents on the Sound for food make this region high priority for harmful algal bloom (HAB) monitoring.

Acknowledgments

We gratefully acknowledge the work of Michael Satchwell for the protein phosphatase inhibition and ELISA assays, Lance Westing and the 2009 Kotzebue High School Field Biology class for sample collection. The authors would like to acknowledge the financial support of the John D and Catharine T MacArthur Foundation and Alfred P Sloan Foundation grants to the Encyclopedia of Life, the National Science Foundation Data Net Program grant number 0830976, the Native Village of Kotzebue and the United States Environmental Protection Agency IGAP grant. Clough's portion of the work was supported by the National Science Foundation while working at the Foundation.

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Project MIDTAL (MIcroarrays for the Detection of Toxic ALgae): final workshop report

Detection of harmful algae in phytoplankton samples using light microscopy is difficult because it requires considerable taxonomic expertise and time to screen samples. Moreover, electron microscopy is required for the correct identification of several harmful species. Accurate identification is crucial because mistakes, in the case of false-negatives, can have serious consequences ranging from serious health problems for people and collapsing regional seafood markets as a result of the ensuing panic, and, in the case of false-positives, fishermen are upset if they are forced to stop their activities unnecessarily. New technology is urgently needed to render detection of large numbers of HAB-causing species more accurately, more cheaply and more rapidly.

One promising possibility is the adoption of microarray technology for detection of HAB species using taxon specific probes. DNA-microarrays are aminosilane-coated glass slides onto which a large number of fluorescently labelled DNA-probes can be spotted. Each of these probes can be designed in such a way that it is specific against the ribosomal RNA of a particular HAB-taxon. If that taxon is present in an environmental sample, then the probe will hybridize with the ribosomal RNA of the taxon and the hybridised RNA/probe complex will become fluorescent when excited by UV-blue-light. Probes can be designed in a hierarchical way, i.e. nested sets of probes against nested sets of natural clades. This improves accuracy and greatly reduces the chances of false positive or false negative assignments.

The European 7th Framework Program project MIDTAL (*MIcroarrays for the Detection of Toxic Algae*) developed a microarray for the detection of toxic species and another one for the detection of their toxins. The main objectives of the project were to demonstrate proof of concept of 'microarray' technology for HAB species detection and to develop this concept into a product for commercialization. The MIDTAL team, coordinated by Prof. Linda Medlin (MBA, Plymouth, UK), included nine partners from seven European countries (Table 1) Each partner focused on a different group of HAB-causing organisms (Table 1), designing DNA probes against these taxa, and testing the probes against monoclonal cultures and environmental samples in which the target species were present. Probes were developed against taxon-specific target regions in the nuclear rRNA encoding gene regions (18S and 28S) because these RNAs are abundant in the ribosomes of all cells. Moreover, the availability of a large database that includes sequences from species across planktonic diversity enables the design of highly specific probes.

After three years work, the MIDTAL partners have achieved their objectives. The resulting MIDTAL microarray target species belonging to the major HAB groups, including toxic dinoflagellates (*Alexandrium, Dinophysis, Azadinium, Gymnodinium, Karenia, Karlodinium, Prorocentrum*), raphidophytes (*Heterosigma*), prymnesiophytes (*Prymnesium, Chrysochromulina*), Dichtyocophyceae (*Pseudochattonella*) and the diatom genus *Pseudo-nitzschia*. In addition, Katrina Campbell (Queen's University, Belfast, UK), developed a microchip based on immuno-detection for the direct and multiplexed identification of saxitoxins, domoic acid and okadaic acid in environmental samples.

To demonstrate their achievement, the MIDTAL partners organized a two-day 'hands on' workshop at the Stazione Zoologica Anton Dohrn in Naples (16-17 April, 2012) at which the microarray was presented to the scientific community. Interested stakeholders from the public and private sector from all over Europe (Italy, Spain, France, Ireland, Croatia, Greece) and beyond (Chile and Tunisia) took the opportunity to familiarize themselves with this sophisticated but simple technique. Participants were grouped in small teams and trained by MIDTAL post-docs and researchers to maximise hands-on engagement. The teachers (Lucia Barra, Eva Pérez Blanco, Simon Dittami, Johannes Hagström, Francisco Hernandez, Jessica Kegel, Gary McCoy, Sara McNamee and Joe Taylor) provided the theoretical background and practical details and tips on bench procedures. All participants were keenly interested in applying the microarray technique to environmental studies if the chip were commercially available and suggested that further developments should include the design and incorporation of

Table 1. MIDTAL team

Principal Investigators	Institution	HA species targeted
Linda Medlin (Coordinator)	Marine Biological Association of the UK, Plymouth (UK)	*Alexandrium, Pseudo-nitzschia, Dinophysis*
Wiebe Kooistra, Marina Montresor	Stazione Zoologica Anton Dohrn, Naples (Italy)	*Pseudo-nitzschia*
Edna Graneli	Linnaeus University, Kalmar (Sweden)	*Heterosigma, Karenia, Gymnodinium*
Beatriz Reguera	Spanish Institute of Oceanography, Vigo (Spain)	*Alexandrium, Dinophysis*
Robin Raine	Martin Ryan Institute, Galway (Ireland)	*Chrysochromulina*, *Prymnesium*
Bente Edvardsen	University of Oslo (Norway)	*Pseudochattonella*
Jane Lewis	University of Westminster, London (UK)	*Alexandrium, Prorocentrum*
Katrina Campbell	Queen's University, Belfast (UK)	Toxin microchip
Yolanda Pazos	INTECMAR (Spain)	*Gymnodinium, Alexandrium,, Dinophysis, Pseudo-nitzschia, Gonyaulax*

Fig 1. Members of the MIDTAL consortium and participants in the MIDTAL Workshop at the Stazione Zoologica Anton Dohrn, Naples, 16-17 May 2012.

probes against toxic species not yet included on the microarray (e.g., palitoxin-producing *Ostreopsis* and ciguatoxin-producing *Gambierdiscus*). The application of the toxin microchip was also presented to the participants during the workshop.

A manual "*MIDTAL: A protocol for a successful Microarray Hybridisation and Analysis*" will be available soon from Koeltz Publishing Co. The microarray produced as a final outcome will be patented and commercialized; negotiations are in progress and the commercial kit will be distributed through the new start-up company MICROBIA in Banyuls sur Mer, France. Further details will be made available through the Midtal website www.midtal.com.

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NEW: "Assessment and management of biotoxin risks in bivalve molluscs"

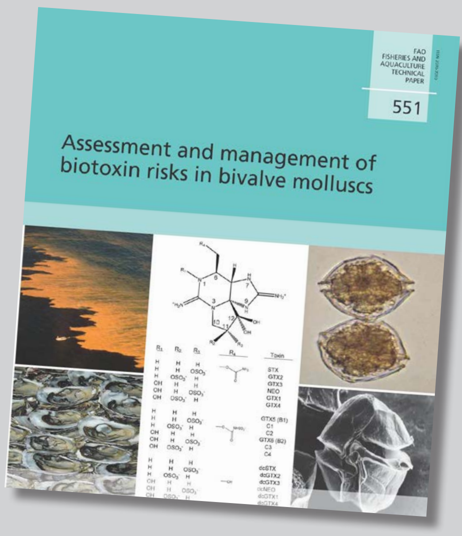
At its 25th session the Codex Committee on Fish and Fishery Products (CCFFP) requested the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to provide scientific advice on biotoxins in conjunction with its work for developing Standards for Live and Processed Bivalve Molluscs.

The CCFFP elaborated further the following specific questions to be covered through this advice:

- Provide scientific advice to the CCFFP to enable the establishment of maximum levels in shellfish for shellfish toxins.
- Provide guidance on methods of analysis for each toxin group.
- Provide guidance on monitoring of biotoxin-forming phytoplankton and bivalve molluscs (including sampling methodology).
- Provide information on geographical distribution of biotoxin-forming marine phytoplankton.

FAO, WHO and the Intergovernmental Oceanographic Commission of UNESCO (IOC) agreed to organize an Expert Consultation to address this request. The group met several times and after some delay the report is finally published:

The following publication/document has been included in the FAO Corporate Document Repository:
http://www.fao.org/docrep/015/i2356e/i2356e.pdf
http://www.fao.org/docrep/015/i2356e/i2356e.zip



ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors (WGBOSV)

The WGBOSW met from 12th to 14th March, 2012 at the European Maritime Safety Agency (EMSA) in Lisbon, Portugal with Brian Elliott (EMSA) as host and Tracy McCollin (United King-dom) as chair. In total there were 39 participants representing 19 countries and EMSA over the course of this meeting and the joint meeting on the morning of the 14th March with the Working Group on Introductions and Transfers of Marine Organisms (WGITMO).

The meeting covered a range of objectives, the group were updated on the status of shipping vector research, discussed methods and procedures used at ballast water treatment test facilities, risk assessment and pathway management methodologies.

Two of the Terms of Reference were completed during a sub-group meeting of WGBOSV in London from 26-27th January, 2012. The sub group met directly before a meeting of the Bulk Liquids and Gases (BLG) subcommittee meeting at the IMO to discuss proposed guidelines on ballast water sampling and analysis. The outcome of the group's discussions was then provided to the BLG meeting the following week by the chair (Tracy McCollin) representing ICES. The approach taken at the meeting was for each country to provide an update on the status of shipping vector research and other interesting information in the form of a National Report. The main outcome of the meeting was a better understanding of the work being carried out in different countries and where there may be opportunities for collaboration, recommendations regarding what information may be needed to carry out risk assessments and how this relates to the current IMO (G7) guidelines and exemptions and a wide ranging discussion regarding the potential improvements that could be made to the IMO guidelines for approval of ballast water management systems (G8).

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Revised Harmful Algal Event Database HAEDAT launched

A longstanding desire to modernise the joint IOC-ICES-PICES database HAEDAT and to improve database functionality has been made possible through a grant provided by the Government of Flanders (Belgium) to the IOC. The obtained results include a shift to using Google Maps which are displayed at the top of every search (fig.1). The maps are automatically zoomed and centred depending on what they have to display, and they can be moved around by clicking and dragging on them. Clicking on one of the markers refines the search to the relevant grid. Maps are also displayed when showing any event with location data (if exact latitude/longitude is available shows this, otherwise shows where the relevant grids are). If a grid code has bounds specified, those bounds are displayed whenever the grid is displayed. All grids associated with a country are displayed by doing a search for that country (fig.2), Users can download the entire database from the 'Browse' page or their 'Options' page. Problems opening the file properly are caused by a bug in Excel, which can be fixed by upgrading to the latest version.

An interface has been added for checking event data. The administrator can check all records, and national editors can check records from their country.

The interface shows 20 records at a time, and only displays the fields that need checking. One can click on the event name to see all the information associated with an event.

An interface has also been added for checking contact data and allows the administrator to check all records, and national editors can check records from their country.

Other Changes includes that dates are checked for consistency when adding/editing an event. An advanced search by grid has been enabled and browsing by region and year has been enabled, and the 'Browse' page now includes the number of events associated with each link. Passwords in the database have been encrypted to make them more secure, and the codebase has been migrated from PHP4 to PHP5.

The future success and usefulness of HAEDAT now entirely depends on that National HAEDAT focal points enter data on a yearly basis and that those countries that are behind in data upload complete the reporting. The IOC data management team and the IODE Project Office in Oostende hope the improved HAEDAT will be well received by data providers and users.

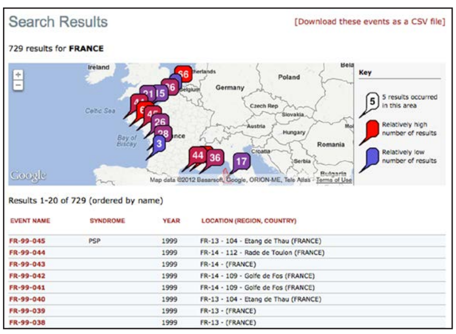

Fig. 1

Fig. 2

Future events

Marine and Freshwater Toxins, Third Joint Symposium and AOAC Task Force Meeting and LC-MS/MS lab training on lipophilic toxins

18-22 & 25-26 June 2012, Tacoma, WA, USA.

Topics for the Symposium are the lipophilic toxins, the DSP outbreak in USA and Canada, saxitoxins official methods, domoic acid, emerging toxins, ciguatoxins, cyanotoxins and test kits.

Please reserve the following dates for above meeting June 18-22, then June 25-26, 2012 for LC-MS/MS lab training for diarrheic and other lipophilic toxins.

As many of you know, the US and Canada experienced their first outbreaks of diarrheic shellfish poisoning (DSP) this year right here in the Pacific Northwest. We will have training for detecting these toxins and a full day devoted to them at the above meeting.

Please contact James Hungerford (James.Hungerford@fda.hhs.gov) if you have interest in these sessions.

IOC Qualifications in Identification and Enumeration of Harmful Microalgae

15-25 August 2012, Copenhagen, Denmark

The course includes 90 hours of teaching and is divided into two parts, each consisting of 45 hours of teaching. The first part of the course is an internet teaching programme, while the second part is a practical workshop in species identification. More information: Jacob Larsen, jacobl@bio.ku.dk.

15th ICHA 2012 Korea
15차 국제 적조 회의
XV International Conference on Harmful Algae

15th International Conference on Harmful Algae

29 Oct-2 Nov 2012, Changwon Gyeongnam, Korea

HAB2012 aims to address all issues related to the causes and effects of marine and freshwater harmful micro- and macro-algae and to serve as a forum for exchanging research outcomes and relevant ideas between researchers, industries, governments and interested parties. More information: www.hab2012.kr

9th International Conference on Molluscan Shellfish Safety

17-22 March 2013, Sidney, Australia

The Call for Abstracts now open. Abstracts should focus around the following themes:Harmful Algal Blooms and Marine Biotoxins, Microbiological and Chemical Risks, Emerging risks from 'Non Bivalve' Molluscs, Risk Assessment and Management, Hazard Inactivation and Elimination Strategies, Production Area Remediation, and Cyanotoxins. ABSTRACT SUBMISSION DEADLINE – THURSDAY 14 JUNE 2012. To submit your abstract please visit www.ICMSS2013.com

ICES-IOC-IMO Working Group on Ballast and Other Ship Vectors (WGBOSV)

18-20 March, 2013, Montreal, Canada

Contact: tracy.mccollin@scotland.gsi.gov.uk

ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

9-12 April 2013 in Vigo, Spain

Contact: Bengt.Karlson@smhi.se

IPHAB-XI: Eleventh Session of the IOC Intergovernmental Panel on Harmful Algal Blooms

22-24 April 2013, Paris, France

Contact: h.enevoldsen@unesco.org

GEOHAB Open Science Conference

25-27 April 2013, Paris, France

Contact: Kudela@ucsc.edu

GEOHAB

Global Ecology and Oceanography of Harmful Algal Blooms

Workshop on "Advances and challenges for understanding physical-biological interactions in HABs in Stratified Environments

This workshop will be held at the Monterey Bay Aquarium Research Institute (MBARI, Moss Landing, California, US) on 21-23 August 2012. The activity is part of the Core Research Project (CRP) on Stratified Systems (GEOHAB, 2008). It is meant to be a platform to develop future international research and, as such, it fits GEOHAB's mandate.

The CRP on Stratified Systems concentrates on the fine-scale distribution of microalgae in stratified environments and their relationships to forcing at larger scales. In stratified environments, specific phytoplankton communities containing HABs have been observed to occur in thin layers. In the last 20 years, investigations have been undertaken to further our understanding of the biological and physical mechanisms involved in the formation, maintenance, and dissipation of thin layers. Research has been focused on the relevance of physical microstructure for fundamental life processes of microorganisms (nutrient and light availability, reproduction, life cycle, ecological interactions) and the relevance of thin layers to HABs. Advances in this area have strongly depended on the development of innovative instruments to observe and adequately sample these environments, as well as advanced numerical models.

The goal of the workshop is to review our current understanding of the processes governing the structure and dynamics of HABs in stratified systems. We aim to identify gaps in our knowledge in order to orient future research for their improved modeling and prediction. The workshop is intended for engineers, physicists, biologists, and modellers, who work - or have worked - on the various aspects of phytoplankton dynamics in stratified systems and can thus contribute to provide a multidisciplinary understanding of this phenomenon. We hope to emerge from this meeting with a conceptual model or 'roadmap' of where biological, physical, and chemical measurements of Harmful Algal Blooms in stratified systems should be headed during the next 10 years, as well as a manuscript synthesizing the findings from this meeting. We are hopeful that this setting will also lead to collaborative proposals to conduct multidisciplinary field experiments addressing this subject. These proposals would most likely involve international collaborations. Given that the workshop will be held in August 2012, the outcomes of the meeting will be ready for the final meeting of GEOHAB (April 2013).

The workshop has been structured in six sessions:

Session 1:
Physical measurements at small scale: instruments, measured parameters, obtained data, low-end versus high-end physical/biological measurements. Plenary speaker: Jules Jaffe.

Session 2:
Biological-physical interactions across scales: the formation, maintenance and decay of thin layers. Plenary speaker: Tim J. Cowles.

Session 3:
Living in a thin layer: calculations of biological rates. Plenary speaker: James M. Sullivan.

Session 4:
Integrating fine-scale measurements into a regional modeling approach. Plenary speaker: Hans Burchard.

Session 5:
Discussion about present gaps, future strategy. Leader: Francisco Chávez

Session 6:
Closed meeting of the organizing committee to prepare the report.

In addition, participants are invited to give an oral presentation or poster. Through presentations and group discussion, we will identify the critical remaining questions, and identify new technologies that may be needed to adequately sample HABs in stratified systems in general and in thin layers in particular.

The Registration and Abstract forms as well as other useful information is found at the GEOHAB webpage (www.geohab.info and http://geohab.aqualight.info/). The registration deadline is June 15th, 2012.

Looking forward to meet you at the MBARI

The Scientific Committee

- Margaret McManus, University of Hawaii at Manoa (GEOHAB)
- Francisco Chávez, MBARI
- Hidekatsu Yamazaki, Tokyo University of Marine Science and Technology
- John Ryan, MBARI
- Oliver Ross, Unitat de Tecnologia Marina, CSIC, Barcelona (GEOHAB)
- Elisa Berdalet, Institut de Ciències del Mar, CSIC, Barcelona (GEOHAB-SSC vice-chair)

The publication of Harmful Algae News is sponsored by the Spanish Institute of Oceanography, Vigo, and the Department of Biology, University of Copenhagen

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ISSN 0020-7918