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THE 16TH INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

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ABSTRACT BOOK

International Society for the Study of Harmful Algae
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KN1 Keynote 1

Harmful algae, a world-wide phenomenon which requires world-wide, multidisciplinary collaboration

Øjvind Moestrup

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The conferences on harmful algae began in 1974 and during the subsequent 40 years a great deal has happened: harmful algae have been found to be much more widespread than initially thought, the number of toxic species has kept increasing and the species to be spreading, often due to human activity. Much has been accomplished during the 40 years, but a surprisingly high number of problems remain unresolved. In fact one of the most basal problems, how to define and identify a species, is approaching a situation resembling chaos and anarchy. This question, which is crucial in monitoring, has –if anything – become more difficult to answer. A species can be defined using morphological, molecular, biochemical or other characters, but the data do not always result in a simple, straight forward answer. For the general public this is hard to understand, and one wonders whether the species concept is slowly developing into being an academic question solely. During my talk I will address some of the problems encountered with identification of species in the different taxonomic groups of algae. Some species are presently relatively easy to identify, but detailed studies on such species often result in additional species being described, which can be very difficult or nearly impossible to distinguish without access to very sophisticated equipment. Can this problem be overcome, or do we have to think in new ways? The genes involved in the production of toxins may play a role in future monitoring for harmful species but such genes have been identified in only two groups of HABs. Fish-killing species have been particularly difficult to work on and with, and despite half a century of work, the information on toxins and toxicity often remain inconclusive. In addition, many of the fish-killing species are difficult to identify by microscopy.
Studies on HABs are multidisciplinary and require collaboration not only between researchers with very different backgrounds but also across countries. I have been asked also to briefly mention the activity and contributions of the IOC Centre on harmful algae in Copenhagen, established 1995, in the dissemination of knowledge on harmful algae, in particular on taxonomy, identification, isolation and culturing.

P1 Plenary 1

New approaches to understanding and predicting harmful algal blooms – what can studies of cyanobacteria teach us?

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Cyanobacterial blooms are a common feature of eutrophied lakes and reservoirs, and occur episodically in estuarine and marine environments. Models of cyanobacterial growth are widely used as a predictive tool to aid in the management of algal blooms in aquatic systems, however there is an acknowledgement that the state variables for models have changed little for decades, and models often have poor predictive power. Current experimental research, using new molecular tools and techniques, combined with traditional physiological and ecological studies, is providing new insights into physiological strategies used by algae. For example, in recent years, studies of strains of individual cyanobacterial HABs have shown that there is considerable variability in physiological responses to environmental conditions, including production of toxins. This has important implications for water quality monitoring and prediction. However, this information in typically not captured in algal growth models. Another insight using new molecular tools and techniques is an understanding of the response of cyanobacterial species to fluctuating nutrient conditions. This information is needed to determine why some species gain a competitive advantage over others. Improvements to model predictions for HABs is therefore most likely to be achieved by embracing new molecular approaches and integrating this with ecological studies and modelling.

P2 Plenary 2

Can evolutionary ecology help us to design better biotoxin detection tools? The case of *Alexandrium*

Shauna A. Murray

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An increase in the occurrence, frequency and severity of blooms of *Alexandrium* species producing saxitoxins (STXs) over the past 30 years has been reported worldwide, including in Australia and New Zealand. Due to their effects on disparate groups of marine organisms and their subsequent role in structuring ecosystems, STXs have been considered 'keystone metabolites'. There have been many hypotheses of the role of STXs for *Alexandrium* species: of these, a role as defensive compounds against predation has been widely investigated, and generally supported. This hypothesis would suggest that predation may have acted as a selective force in the evolution of toxicity in the genus. This talk will highlight the potential role of STX in the diversification of *Alexandrium*, in particular, the phylogeny of the *sxt* gene cluster and the evolution of novel and cryptic species of *Alexandrium*.
I will discuss the application of such genetic information to the design of rapid detection tools for marine biotoxin monitoring, based on the gene *sxtA*. Such tools have now been applied to quantify blooms of *Alexandrium tamarense* complex species and *Alexandrium ostenfeldii* from Australian, New Zealand and European sites, and have been successfully used as a rapid screen for the presence of STX in commercial oyster species.

P3 Plenary 3

Marine toxin detection methods and preparation of diarrhetic shellfish toxins (DSTs) and paralytic shellfish toxins (PSTs) by large algal culture and chemical conversions

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Maine toxins in shellfish have been tested by the mouse bioassay (MBA) in many countries including Japan. Recently several alternative testing methods have been developed. The most widely accepted method is liquid chromatography (LC) with mass spectrometry (MS) or fluorescent detection (FD) because these methods are superior to the MBA in sensitivity and accuracy. Accurate calibration standards are essential elements to validate instrumental methods. Several certified reference materials are available from the National Research Council of Canada (NRC) Biotoxin Metrology Program. This program has contributed to a worldwide routine monitoring of marine toxins. On the other hand, several calibration standards of shellfish toxins have been prepared in our research project in Japan. Okadaic acid (OA) and dinophysistoxin-1 (DTX1) are prepared from *Prorocentrum lima*; or *Dinophysis* spp.; yessotoxin (YTX) from *Protoceratium reticulatum*, and pectenotoxin-2 (PTX2) from *Dinophysis* spp. Suitable strains were selected and the culture conditions were optimized. To prepare paralytic shellfish toxin (PST) calibration standards, C1 and C2 toxins produced by the cyanobacteria *Anabaena circinalis* were used to chemically produce GTX2/3 (HCl), GTX5 (DTT), and dcGTX2/3 (heat at pH 7.0). GTX5 was further converted to dcSTX by hydrolysis. GTX1/4 were produced by large cultures of *Alexandrium tamarense*. The reductive elimination of 11-O-sulfate group in GTX1/4 by DTT yielded neoSTX. All reaction conditions were optimized, to enable the production of the major PSTs starting from cultured cells.

P4 Plenary 4

Risk assessment of seafood toxins

Rex Munday

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Many marine organisms produce complex secondary metabolites, some of which are highly toxic to mammals. Such organisms are important food sources for marine animals, and, when ingested, the toxins that they contain may be absorbed and stored in the tissues of the predators. These may then poison animals higher up the food chain, and many cases of human intoxication of seafood consumers are reported each year.
In order to alleviate the likelihood of such intoxication, assessment of the risk of seafood toxins is required so that levels of such toxins that are unlikely to cause adverse effects in humans can be identified. Regulatory limits for such toxins can then be put in place.
The primary objective of risk assessment is the establishment of No Observable Adverse Effect Levels (NOAELs), defined as the highest doses at which no adverse effects of the toxins are observed. By application of suitable safety factors, the NOAELs may be used to estimate the amount of toxin that can safely be consumed by humans.
While good progress has been made in the risk assessment of seafood toxins, some problems still remain. These will be discussed, with particular reference to recent work on the risk assessment of saxitoxin derivatives.

P5 Plenary 5

Biogeography and toxicity of *Gambierdiscus* species

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The genus *Gambierdiscus* was first described by Adachi and Fukuyo in 1979 from material gathered in the Gambier Islands, French Polynesia. The genus was considered monotypic (*G. toxicus*) until Faust discovered *G. belizeanus* from the western Caribbean in 1995. Soon Holmes, in 1998, added *G. yasumoitoi* from Pulau Hantu (Singapore) and in 1999 the brilliant paring of Chinain and Faust (molecular biologist and morphologist) provided descriptions of *G. australes* (Australes Archipelago, FP), *G. pacificus* (Tuamotu Archipelago, FP) and *G. polynesiensis* (Australes and Tuamotu Archipeligos, FP). Their SEMs and morphological descriptions were substantiated by sequence data. In 2009, Litaker working with Faust and colleagues revised the genus including four new species from Belize, *G. caribaeus*, *carolinianus*, *carpenteri* and *ruetzleri*. This study provided light micrographs and SEMs for all described species and added the first sequence data for *Gambierdiscus* to GenBank. The emergent species suite was joined by *G. excentricus* described by Fraga in 2011 from the Canary Islands (NE Atlantic) and *G. scabrosus* from Japan described by Nishimura and colleagues in 2014. The dynamic state of biogeographical, physiological and toxicological research on *Gambierdiscus* has been greatly assisted by the availability of sequence information and PCR assays. These allow rapid identification of taxa, facilitate comparisons of species suites from different locations and habitats and extend the known range of many species. With questions of identification, distribution and abundance in hand, sorting out the toxicity among *Gambierdiscus* species (isolates) is the next, crucial data layer in a functional biogeography of the genus.

P6 Plenary 6

Ten years of *Didymosphenia geminata* in New Zealand

Cathy Kilroy

The stalked freshwater diatom *Didymosphenia geminata* has gained notoriety over the past 25 years as a significant nuisance algal species in rivers and lakes. *D. geminata* produces massive proliferations of carbohydrate stalk material in waters with very low nutrient concentrations. As well as negatively affecting the aesthetic and recreational values of rivers, *D. geminata* blooms change river ecosystems and can have direct economic impacts (e.g., on irrigation). The range of *D. geminata* blooms in the Northern Hemisphere has been expanding since the early 1990s, but the discovery of blooms in a South Island, New Zealand, river in October 2004 raised the profile of *D. geminata* as a global issue. *D. geminata* was considered to be a new introduction to New Zealand, and was assigned "unwanted organism" status, because of its potential to degrade low-nutrient, pristine rivers. An NZ-Government-backed research and management response continued until 2008, when it was replaced by regional management. The New Zealand response reinforced a view that *D. geminata* blooms worldwide were caused by the rapid human-mediated spread of an invasive variant, especially in affected Northern Hemisphere areas where *D. geminata* was known to be present historically. Ten years after its first discovery in New Zealand, understanding of this diatom has changed. The results of experiments in New Zealand support a new view that *D. geminata* blooms in the Northern Hemisphere may be caused by environmental change: specifically, declining inorganic phosphorus (DRP) in rivers. In New Zealand, however, *D. geminata* is almost certainly non-indigenous. It has flourished because some South Island rivers provide apparently perfect conditions for blooms – in particular, low DRP (< 2 ppb). Conversely, elevated DRP generally corresponds with the absence of blooms. High DRP is also likely associated with failure to detect even low levels of *D. geminata* in some areas, including the North Island. I trace the history of *D. geminata* in New Zealand, including evidence supporting its assumed non-indigenous status here, and highlights of what we have learned over the past 10 years about its biology, distribution and impacts.

KN2 Keynote 2

Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change

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Harmful (toxic, food web altering, hypoxia generating) cyanobacterial blooms (CyanoHABs) are proliferating world-wide due to anthropogenic nutrient enrichment, and represent a serious threat to the use and sustainability of freshwater and coastal resources. Traditionally, phosphorus (P) input reductions have been prescribed to control CyanoHABs, because P limitation is widespread and some CyanoHABs can fix atmospheric nitrogen (N_2), thereby satisfying their nitrogen (N) requirements. However, eutrophying systems are increasingly plagued with non N_2 fixing CyanoHABs that are N and P co-limited or even N limited. In many of these systems N loads are increasing faster than P loads. Therefore N and P input constraints are likely needed for long-term CyanoHAB control. Climatic changes, specifically warming, increased vertical stratification, salinization, and intensification of storms and droughts, play additional, interactive roles in modulating CyanoHAB frequency, intensity, geographic distribution and duration. In addition to having to consider reductions in N *and* P inputs, water quality managers are in dire need of effective tools to break the synergy between nutrient loading and hydrologic regimes made more favorable for CyanoHABs by climate change. Tools available for countering CyanoHABs include; 1) altering the hydrology (enhance flushing rates, when feasible), 2) induce de-stratification, 3) decrease nutrient fluxes from sediments by removing the sediments or capping sediments with clay, and 4) apply "cyanocides" such as peroxide. Each of these has its limits and pitfalls. Future CyanoHAB management approaches must incorporate both N and P loading dynamics within the context of altered thermal and hydrologic regimes associated with climate change.

P7 Plenary 7

Researchers as partners to the seafood industry and risk managers in developing appropriate risk management of harmful algal blooms

Alison Turnbull

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When harmful algal blooms occur in areas that are used by wild harvest fisheries or aquaculture they can have devastating effects; commercially and on human health. Industry and regulators have a combined role in managing the risks to consumers and to future trade and market access. Recent developments at Codex have placed increased attention on non-traditional vectors for marine biotoxins. In Australia this has heightened due to the detection of paralytic shellfish toxins in significant levels in abalone during a bloom of *Gymnodinium catenatum*, (causing an industry estimated loss of $1.38 million AU). Further to this an unprecedented bloom of *Alexandrium tamarense* impacted multiple fishing industries in Tasmania; the estimated direct costs were $6.3 million AU.
Several significant knowledge gaps that hampered the risk assessment/risk management processes during these events were: the difficulty in identifying and enumerating *A. tamarense*; the accumulation potential of saxitoxin analogues in various tissues of the non-traditional vectors such as rock lobster and abalone; the unknown consumption pattern of these tissues; a novel saxitoxin analogue; uptake and depuration mechanisms and rates; and the potential use of sentinel species for management. Underlying these knowledge gaps for the non-traditional vectors was the question of whether consumers were really at risk.
The role of risk analysis, and in particular of researchers in filling short-term (incident response), mid-term (addressing information gaps to underpin appropriate risk management by industry and regulators) and long-term (improving ongoing risk management) needs will be discussed, using the Australian events as examples. The benefits of a partnership approach to managing food safety and trade and market access issues involving industry, regulators, and researchers, as facilitated by SafeFish in Australia, will be detailed.

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S1.1

Formal revision of the *Alexandrium tamarense s*pecies complex (Dinophyceae) taxonomy: The introduction of five species with emphasis on molecular-based (rDNA) classification

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The *Alexandrium tamarense* species complex is one of the most studied marine dinoflagellate groups due to its ecological, toxicological and economic importance. Several members of this complex produce saxitoxin and its congeners - potent neurotoxins that cause paralytic shellfish poisoning. Isolates from this complex are assigned to *A. tamarense*, *A. fundyense*, or *A. catenella* based on two main morphological characters: the ability to form chains and the presence/absence of a ventral pore between plates 1′ and 4′. However, studies have shown that these characters are not consistent and/or distinctive. Further, phylogenies based on multiple regions in the rDNA operon indicate that the sequences from morphologically indistinguishable isolates partition into five clades. These clades were initially named based on their presumed geographic distribution, but recently were renamed as Groups I-V following the discovery of sympatry among some groups. In this study we present data on morphology, ITS/5.8S genetic distances, ITS2 compensatory base changes, mating incompatibilities, toxicity, the *sxtA* toxin synthesis gene, and rDNA phylogenies. All results were consistent with each group representing a distinct cryptic species. Accordingly, the groups were assigned species names as follows: Group I, *A. fundyense;* Group II, *A. mediterraneum;* Group III, *A. tamarense*; Group IV, *A. pacificum;* Group V, *A. australiense*.

S1.2

Population genetic studies of worldwide populations in the toxic dinoflagellate *Alexandrium catenella* (Dinophyceae) by use of haplotype and microsatellite markers

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The distribution of *Alexandrium catenella* has been increasingly documented worldwide during the last decades. To examine the genetic relatedness of global populations, we conducted a genetic study using 2 haplotype markers and 7 microsatellite markers. Seawaters or sediment samples were collected from 11 different localities (17 samples) along 10 Japanese, 3 Chinese, 2 Mediterranean Sea, 2 New Zealand coastal waters and 839 clonal strains (temperate Asian clade) were analysed. One of the two haplotype markers showed high resolution to discriminate the difference of the geological origin in the populations. We could obtain the sequences from 673 clonal isolates. Two haplotypes were predominant of 85 haplotypes. The first one "*Haplo01*" (n = 316 isolates) and the second one "*Haplo02*" (n = 122) were predominated by Japanese isolates and Chinese isolates, respectively. Some Japanese isolates also belonged to *haplo2*. Interestingly, the Sea of Japan population had unique and highly diversified haplotypes derived from *haplo2*. New Zealand populations were mainly divided into three groups, i.e. the first one belonged to *Haplo2*, the second one derived from *Haplo1*, and the third one positioned in the middle of *Haplo1* and *Haplo2* groups. The half of Mediterranean Sea isolates belonged to *Haplo1* and other half isolates had unique haplotypes derived from *Haplo2*. Microsatellite analysis data showed the significant population differentiation among most of the pairwise populations except for several Japanese pairwise populations. Results of the haplotype analysis well supported those at microsatellites analyses.

S1.3

Assessment of the use of NGS metabarcoding for the identification of toxic benthic dinoflagellates from environmental samples

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The identification of toxin-producing dinoflagellates for risk assessment and monitoring programmes requires considerable taxonomic expertise. It can be difficult to morphologically differentiate toxic and non-toxic strains and the isolation and culture of isolates is also very labour intensive. Recent advances in molecular methods provide promising tools for assessing diversity in environmental samples. To date most molecular methods utilise species-specific assays for rapid and accurate idenfication, however these methods are limited in the number of samples and species that can be targeted. As sea water temperatures continue to rise the distribution of toxic species is changing and therefore, regionally, species of interest are also changing. For example, cells from the sub-tropical genus *Gambierdiscus*, were recently detected in temperate New Zealand waters for the first time. We sought to develop a universal metabarcoding tool based on next-generation sequencing (NGS) for the identification of dinoflagellates from environmental samples. Benthic and epiphyte dinoflagellates were collected using various methods from sites in Northland, New Zealand. We investigated dinoflagellate diversity using barcode sequences (18S ribosomal RNA, 28S ribosomal RNA, and mitochondrial cytochrome b genes) generated by Illumina NGS technology. We compared molecular data with microscopic analyses and toxin data. The use of NGS metabarcoding shows great potential as a tool for the identification of toxic dinoflagellates from environmental samples.

S1.4

DNA analysis of ballast and port sediments collected over 26 years provides unique insights into the transportation and distribution of marine eukaryotic species

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Shipping traffic continues to be a major contributor to the global spread of potentially harmful marine microorganisms. Currently, large-scale biological monitoring of ship ballast water and shipping-port sediments is hindered by time and financial constraints. Metagenomics is a recently developed molecular method that identifies multiple taxa concurrently using genetic databases and offers parallel processing of many environmental samples, greatly reducing the time and costs associated with large-scale biological surveys. We have applied metagenomic methods to a unique sample series, consisting of 26 international ballast tank sediments and 36 Australian port samples, collected over a timeline of almost 30 years (1988-2014). This dataset will provide valuable insights into the movement and distribution of eukaryotic marine species along Australian coastlines as well as highlight the role of ships' ballast in species movement. This study demonstrates the value of metagenomics as a tool for monitoring and managing the spread of environmentally and economically destructive marine organisms.

S2.1

The dynamics of sexual phase in the marine diatom *Pseudo-nitzschia multistriata*

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Sexual reproduction is a fundamental phase in the life cycle of diatoms, linked to the production of genotypic diversity and the formation of large-sized initial cells that ensure population persistence. Only cells below a certain threshold size can be sexualized, but environmental factors can tune the success of sexual reproduction. We investigated the time course of the sexual phase in the marine heterothallic diatom *Pseudo-nitzschia multistriata* in confocal laser scanning and time lapse microscopy, focusing on gamete formation, conjugation and the development of auxospores. The movement of chloroplasts during gametogenesis has an important role for the correct accomplishment of the process. We also investigated the role of endogenous (cell density and physiological conditions of parental strains) and exogenous (role of water mixing) factors for the success and timing of sexual reproduction. Successful sexual reproduction can only be achieved when crossing parental strains in exponential growth and the formation of gametes occurs when a threshold cell concentration is achieved. Moreover, the onset of the sexual phase was coupled to a marked reduction of growth of the vegetative parental cells. The crosses carried out in physically mixed conditions produced a reduced number of sexual stages as compared to crosses in still conditions, showing that mixing impairs sexualisation. The results of our experiments suggest that 1) gametogenesis is a tightly regulated process that may involve a series of checkpoints; 2) the signalling that triggers the sexual phase is favoured when cells can accumulate, facilitating contacts and/or the perception of chemical cues.

S2.2

The possible effects of *Calanus* copepods on domoic acid production and chain formation of the toxic diatom *Pseudo-nitzschia seriata*

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The possible effects of presence of *Calanus* copepods, *C. hyperboreus* and *C. finmarchicus*, on domoic acid production and chain formation of the toxic diatom *Pseudo-nitzschia seriata* were studied. The results show that toxicity of *P. seriata* cells increased in the presence of the copepods and that this induced response was chemically mediated without direct physical contact between the organisms, suggesting that potential waterborne cues from the copepods or changes in water chemistry, i.e. some chemical signal, induced the response in the *P. seriata* cells. Domoic acid may play a role in defense against grazing in *P. seriata*. To test if the possible chemical cues from the damaged *P. seriata* cells alone would induce domoic acid production in the grazing experiment we also exposed live *P. seriata* cells to *P. seriata* cell homogenate. This was not the case. Chain formation of *P. seriata* was not affected without physical contact with the copepods and neither by the *P. seriata* cell homogenate. The proportion of single cells of *P. seriata* seemed to increase when the copepods grazed directly on the cells, but whether this was due to selective grazing on chains or suppression of chain formation due to potential chemical cues released by the copepods, could not be distinguished.

S2.3

Proliferation and fertilization cell cycles in the toxic dinoflagellate *Alexandrium minutum*: Two not so different processes

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Eukaryotic cells enter the cell cycle through two distinct processes: fertilization and cell proliferation. Cell proliferation is the process whereby cells reproduce themselves by growing and then dividing into two equal copies. In dinoflagellate species with a zygotic life cycle, haploid cells are able to combine with other haploids by fusion of two cells and formation of a diploid zygote able to transform into a resting cyst. In HAB ecology, the control of these two processes is the key aspect to determine the magnitude, recurrence and consequences of HABs events. We have used clonal cultures of the toxic dinoflagellate species *Alexandrium minutum* to study the complete cell cycle at both the ploidy, morphological and chromosomal level using Imaging Flow Cytometry and Fluorescence In Situ Hybridization (FISH). Our results characterize asexual and sexual stages in all the different phases of the cell cycle and show that the length of the G2 phase may be modulated by the environmental nutrient status. The study of the sexual cycle first shows undisturbed dinoflagellate zygotes following a dividing cycle of similar characteristics than the vegetative cycle, i.e., with division during the dark period, and encystment as just an alternative process. Therefore, *A.minutum* is able to proliferate also through fertilization. These results clarify the role and relevance of sexuality in the life cycle of dinoflagellates and open new possibilities for the characterization at a given time of the proliferation and fertility status of the bloom.

S2.4

The HABs-forming dinoflagellate *Akashiwo sanguinea* produces resting cyst

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The dinoflagellate *Akashiwo sanguinea* is a well known, cosmopolitan harmful microalga which frequently forms harmful algal blooms (HABs) in marine estuaries from temperate to tropical waters and has posed a severe threat to fish, shellfish, and sea birds. Therefore, it is of vital importance to understand the ecology, particularly the mechanisms regulating its ubiquitous geographic distribution and frequent recurrence of blooms, of the species. To date, the mechanisms regulating distribution and recurrence of HABs by this species have been poorly understood. While resting cyst production can play a central role in the geographic expansion and initiation of HABs, studies of the life cycle of this alga, including cyst production, have been absent. The presentation will demonstrate that *A. sanguinea* can produce resting cysts homothallically and sexually, and present evidence for cell pairs in sexual mating, biflagellated planozygote formation, cyst production, and time series for germination of cysts to germlings with two longitudinal flagella. Phylogenetic analysis of LSU rDNA sequences revealed a monophyly of this species and thus possibly a recent common ancestor for all global populations. The discovery of resting cyst production by *A. sanguinea* suggests its frequent recurrence of blooms and global distribution may have been facilitated by the natural and anthropogenic transport of resting cysts such as transportation via ships' ballast water.

S3.1

Frontiers of HAB research in the Arctic: towards defining emerging patterns of biogeography and biodiversity associated with regime shifts in coastal systems

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Global change mediated by sea temperature rise, enhanced ice melting and other anthropogenic influences is predicted to have a profound influence on Arctic coastal regions. This has led to speculation regarding differential effects on biogeography and diversity of HABs and associated toxins. We conducted an expedition (ARCHEMHAB) to west Greenland with transects across the Irminger Sea to fjord systems in northwestern Iceland. In west Greenland, longshore sampling was accompanied by fjord transects to the edge of the glacier ice shelf. Field observations included physical oceanographic parameters (CTD) and bio-optical profiles, vertical net tows and Niskin bottle sampling (plankton, nutrients, pigments, dissolved organic matter), and benthic sediment grabs for dinoflagellate cysts (*Alexandrium* spp.) and bivalve molluscs. We found numerous HAB taxa (*Alexandrium, Azadinium/ Amphidoma*, *Dinophysis*, *Protoceratium reticulatum*, *Pseudo-nitzschia* spp.) and associated toxins, primarily gonyautoxins, spirolides C and des-methyl C, and domoic acid, with only trace levels of dinophysistoxins, pectenotoxins and yessotoxin, in the plankton from the water column. Community analysis of plankton size fractions by DNA bar-coding based upon sequencing the LSU rDNA D1/D2 region revealed comparative patterns of diversity for Disko Bay (Greenland) versus stations in an Icelandic fjord. The high species diversity in the microplankton fraction on the Greenland coast showed a strikingly high representation of cryptic dinoflagellate taxa. No definitive linkages to HAB-relevant regime shifts or environmental driving forces such as glacial melting on population dynamics were defined, but such multi-faceted expeditions provide the basis for future scenarios and modelling change processes of HAB distribution in Arctic waters.

S3.2

Adaptive potential of geographically and genetically differentiated Baltic populations of the toxic dinoflagellate *Alexandrium ostenfeldii*

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Phytoplankton populations from marine and freshwater systems often show high levels of genetic diversity. When reflected by phenotypic variability such standing genetic diversity may help populations to cope with environmental changes. In this study we examine the relationship between genetic diversity and phenotypic variability in the response of four Baltic *A. ostenfeldii* populations to climate related salinity and temperature changes. Multiple genotyped strains grown from cyst beds of bloom sites located in different Baltic temperature and salinity regimes were exposed to salinity and temperature gradients reflecting predicted and extreme conditions of climate change. Growth rates, toxicity and bioluminescence emission were determined as response parameters. For all measured traits a significant effect of genotype on the response to temperature and salinity was identified. Responses were both selective and plastic. Strain variability buffered the net- or population specific responses, and differences in growth, toxicity and bioluminescence were only significant at the environmental extremes. Some population specific effects were observed on specific traits. The results of this experiment suggest that the gene pool of all studied Baltic *A. ostenfeldii* populations is diverse enough to ensure adaptation to changing temperature and salinity conditions.

S3.3

Australian dust storm associated with extensive *Aspergillus sydowii* fungal "bloom" in coastal waters

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Dust from deserts not only deposit nutrients into the oceans that can enhance primary production, but also spread microbial communities. Using continuous plankton recorder technology, we report how in September 2009 an Australian dust storm triggered a massive coastal "fungal bloom" of *Aspergillus sydowii*, stretching over an area 25 times that of the surface of England. While no impacts comparable to Caribbean gorgonian fan coral disease ("aspergillosis") were observed, Australian fungal cultures reduced *Symbiodinium* dinoflagellate coral symbiont motility, thus raising the possibility of future marine ecosystem impacts from more pathogenic strains. Dust storms are likely to increase with climate change as Australia becomes drier. *Aspergillus* mycotoxins such as gliotoxin have been demonstrated to accumulate in shellfish and can cause immunosuppression in animals and humans.

S3.4

Evolution of HAB causative species and possible links during the last five decades in Chinese coastal waters

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A remarkble change of harmful algal composition has occurred during the last half century in Chinese coastal waters. The frequency of harmful algal blooms has shown a remarkable increased trend while size spectrum of blooming species has become much smaller since the 70's of last century. More recently, around 60 HAB events occurred each year in China. Areas with frequent HABs mainly include the Changjiang estuary of the Esat China Sea (ECS), the Zhujiang estuary in the South China Sea (SCS), the Bohai Bay and the Liaodong Bay(the northern sea area of China). Particularly, large scale blooms (over 1 000 km^2) have been recorded every year since 1998. *Prorocentrum donghaiense* has become the recurrent bloom species in the East China Sea for more than ten years.Since 1999, *Phaeocystis globosa* has formed massive blooms in the South China Sea and in Tianjing coastal water of the Bohai Sea. A dinoflagellate bloom, caused by *Cochlodinium geminatum* which has not formed blooms elsewhere around the world, occurred recently in the Zhujiang estuary, SCS. Newly recorded blooms caused by *Karodinium veneficum and Cochlodinium polykrikoides* in the East China Sea are also remarkable. Since 1998, nearly 90 blooms caused by *Karenia mikimotoi* have occurred in China. The massive bloom of this species in the East China Sea in 2005, 2012 resulted in the heavy loss of fish and shellfish farming. *Azadinium poporum*, an AZA toxin producer, was rerecorded in the Bohai Sea, the East China Sea as well as in the South China Sea. More recently, brown tide caused by *Aureococcus anophageferense* has been registered in the near shore of the Bohai Sea. A number of species have been detected for the first time in this area. The possible related factors for the shift of causative species of harmful algae in China coastal waters are discussed.

S4.1

'Toxic in Crowds' – Fine scale variability in microcystin production across a eutrophic lake

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Toxic cyanobacterial blooms are increasing in prevalence globally. Of the known cyanobacterial toxins the hepatotoxic microcystins are the most notorious. Laboratory studies have shown correlations between microcystin quotas and a multitude of physiochemical variables including nutrients, temperature and pH. Whilst these laboratory-based experiments allow environmental conditions to be stringently controlled, studying cyanobacteria in these 'artificial' environments may alter or remove variables that regulate microcystin production. Over the past three years, our group has studied microcystin production in a small eutrophic lake (Lake Rotorua, Kaikoura), using a series of mesocosm experiments and surveys of natural populations. Initial experiments demonstrated a strong correlation between microcystin production and cell density in the mesocosms. In this study, we undertook two extensive profiles of the surface waters across the whole lake, and a further intense study focused on a small enclosed bay to explore whether this same phenomenon occurred naturally with spatial variation in cell density around the lake. Cyanobacterial density was assessed fluorometrically and microscopically, and samples were collected to determine microcystin (liquid chromatography-mass spectrometry) and nutrient concentrations, and to examine expression of genes involved in toxin production. A range of physiochemical variables including temperature, pH and dissolved oxygen were also measured. These data are being used to elucidate how interactions between abiotic and biotic variables influence microcystin synthesis and may ultimately help to predict parts of a lake or periods of greatest risk to health.

S4.2

Production of siderophores by freshwater cyanobacteria: culture and field studies

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Cyanobacteria may require elevated levels of iron for growth in comparison to eukaryotic algal competitors. To meet this need, some cyanobacterial species produce strong ferric iron chelators (siderophores) to facilitate iron uptake in typically oxic environments. In this study, laboratory culture experiments were combined with field surveys to examine whether siderophores are an important iron uptake mechanism for cyanobacteria in eutrophic freshwater systems. N-fixing and non-fixing *Dolichospermum* (also known as *Anabaena*), *Microcystis*, and *Aphanizomenon* isolates from the lower Great Lakes were grown in iron-limited media and tested for siderophore formation using chemical and HPLC-MS techniques. Some, but not all, of the N-fixing *Dolichospermum* strains produced the hydroxamate siderophore schizokinen. Strains of the cyanobacterium *Microcystis* did not produce detectable hydroxamate siderophores in culture. To look for siderophores in natural environments a novel solid phase extraction coupled with a LC-MS analytical protocol was applied to selected sites in Lakes Erie and Ontario. *In situ* production of hydroxamate siderophores was not detected at the >30 locations. These results suggest that siderophore production is a species-specific trait, and that contrary to current theories, it is not an important driver for the harmful cyanobacterial blooms commonly observed in the lower Great Lakes.

S4.3

Isolation and characterisation of a virus infecting the invasive filamentous cyanobacterium *Cylindrospermopsis raciborskii* in the Netherlands

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Viral mortality is likely to influence the abundance and spread of invasive harmful cyanobacteria. In this study, we have discovered and characterised a virus of *Cylindrospermopsis raciborskii,* a potentially toxic species. The virus (CrV) and its *C. raciborskii* host were isolated from a lake in the Netherlands. The 120 kb dsDNA CrV belongs to the *Siphoviridae* family, with an icosahedral capsid around 60 nm wide, and a long non-contractile, flexuous tail with an average length of around 600 nm. We found that viral infection increased the average cell lengths by 30% before cell lysis. As viruses can infect individual cells in a filament, viral infections can fragment colonies into smaller units which may potentially disperse further. With the discovery of CrV, the ability of this virus to control populations of *C. raciborskii* can now be examined in detail, both experimentally and in the natural environment. In particular, examining the effects of environmental factors (e.g. temperature, light and nutrients) on viral infection of *C. raciborskii* will allow insights into the potential spread of the species in the context of global climate change.

S4.4

Exploring the structural and functional diversity of bacterial communities associated to bloom-forming cyanobacteria

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In the global framework of researches dealing with the determinism of cyanobacterial blooms in freshwater ecosystems, a lot of papers have been published in the ten last years on bacterial communities associated to cyanobacteria during bloom events. Several of them provided some evidence of specific interactions between cyanobacteria and heterotrophic bacteria. But the main limitations of these studies are that it is quite difficult (i) to distinguish between the part of environmental parameters and the one due to the cyanobacteria, on the composition of the bacterial communities and (ii) to characterize the functional diversity of these communities. In order to overcome these limitations, we compared the structural and functional diversity of the bacterial communities associated with fifteen strains from the Paris Museum Collection of Cyanobacteria, belonging to four genera (*Anabaena*, *Aphanizomenon*, *Microcystis* and *Planktothrix*). Our pyrosequencing approach has revealed (i) that the bacterial communities associated with all cyanobacterial strains, display a high richness and diversity and share a similar composition with those associated to natural blooms, (ii) no significative differences in regard to the date of isolation of the cyanobacterial strains (from 3 to 11 years) and, (iii) significant differences in the composition and in the functional diversity (Ecoplates approach, Biolog) between the bacterial communities associated to the four cyanobacterial genera. All these findings suggest that specific bacterial communities displaying contrasting capacities to use various carbon sources are associated with the different bloom-forming cyanobacterial species, which raises the question on the nature of interactions occurring between bacteria and cyanobacteria.

S4.5

Change of the toxin profile of *Microcystis aeruginosa* in response to grazer signalling

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Microcystins are hepatotoxic peptides produced by cyanobacteria like *Microcystis* spp. and *Planktothrix* spp. The main structure is characterized by a cyclic peptide consisting of seven amino acids. Therefore, there are known about 90 different variants of this class of cyanotoxins. Each cyanobacteria producing microcystin is defined by its unique profile of 5 to 10 microcystin variants. A change of the toxin pattern can be caused by various stress factors including grazing signals. Even an increase of the total amount of microcystin per cell was observed in *Microcystis aeruginosa* due to the presence of grazers (e.g., *Daphnia* spp.) [1].
Yasumoto et al. (2005) showed that extracted alkyl sulphates of *Daphnia pulex* as well as structure analogues can induce morphological changes in the green alga *Scenedesmus* sp. [2]. With these verifiably bioactive substances bioassays with *Microcystis aeruginosa* were accomplished to induce physiological reactions, namely changes of the toxin profile. Microscale cultures of *M. aeruginosa* have been incubated for several days with extracts of *Daphnia pulex* containing the bioactive sodium alkyl sulphates and sodium dodecyl sulphate as structure analogue. The intracellular extracts were measured with high performance liquid chromatography coupled with triple quadrupole mass spectrometry in multi reaction monitoring (MRM) mode to acquire the relative changes of single microcystins. First results indicate a change of certain toxins in response to the applied alkyl sulphates.

S5.1

Genetic diversity of the toxigenic family Kareniaceae (*Gymnodiniales, Dinophyceae*) in France, with the description of a new potentially toxic Karlodinium species

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The family Kareniaceae is mostly known in France for recurrent blooms of *Karenia mikimotoi* in the Atlantic, English Channel, and Mediterranean Sea and for the unusual green discoloration in the Diana lagoon (Corsica) caused by *Karlodinium corsicum* in April 1994. In terms of diversity, this taxonomic group was long overlooked owing to the difficult identification of these small unarmored dinoflagellates. In this study, thanks to the molecular characterization performed on single cells from field samples and cultures, twelve kareniacean species were assigned to the known genera *Karenia*, *Karlodinium* and *Takayama*, whereas one species could not be affiliated to any described genus. The molecular phylogeny of the D1-D2 region of the LSU rDNA showed that five of them formed a sister taxon of a known species, and could not be identified at species-level on the basis of molecular analysis only. Among these taxa, one *Karlodinium* was successfully cultured which enabled us to investigate the external morphological features (using two procedures for cells fixation), ultrastucture, and pigment composition. The results of our analyses corroborate the genetic results in favour of the erection of a new *Karlodinium* species which possesses an internal complex system of trichocysts connected to external pores particularly abundant in the epicone, and a peculiar pigment composition. In addition, preliminary assays showed a positive haemolytic activity.

S5.2

RADtag sequencing – a reduced representation method for population genomic analyses of HAB-species

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In recent years, research has yielded novel insights regarding population genetic structure and genetic diversity of phytoplankton. These studies have revealed both the presence of large unstructured populations with high gene flow, but also of highly differentiated neighboring populations. However, current methods such as microsatellites and AFLP have substantial limitations for further exploration of the underlying processes. Instead there is a need for population genomics, i.e. simultaneous genotyping of many thousands of loci across the genome, which combines genomic technologies and population genetic concepts. Because many HAB species such as dinoflagellates and raphidophytes have huge genomes, sequencing entire genomes of hundreds of strains is not an option. Instead, an approach that allows for sequencing of many strains, but at a reasonable cost, would be a reduced representation technique. We have identified RAD (Restriction-site Associated DNA) tag sequencing as a potential method for HAB species. We tested standard RAD and 2bRAD for one marine dinoflagellate and one freshwater raphidophyte. We found that RAD sequencing using an 8-cutter enzyme was useful for both species and yielded over 20,000 polymorphic loci (SNPs) at high coverage. 2bRAD, on the other hand was not useful for these species. Results presented will include identification of SNPs using the Stacks software and population genetic summary statistics. Methodological issues including sequence coverage and costs, as well as parameter choice for downstream analyses, will be discussed.

S5.3

The complexities of the marine cyanobacteria *Lyngbya majuscula*: insights from a whole genome analysis

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The toxic benthic cyanobacterium, identified morphologically as *Lyngbya majuscula*; frequently blooms in summer months in Moreton Bay, in subtropical Australia. It has negative effects on seagrass beds and species that rely on them. The recent phylogenetic reclassification of tropical strains of *L. majuscula* to *Moorea producens* and *M. bouilloni* by Engene et al. (2012) has raised questions about the taxonomic status of the species in Moreton Bay. We have sequenced the genome of a Moreton Bay isolate (CS953) and the 16S regions from two collections of benthic cyanobacteria from Moreton Bay. The 16S sequences align closely with *Trichodesmium erythraeum* and cluster with *Hydrocoleum* sp, within a Neighbour Joining phylogenetic tree, and are distinct from *Lyngbya* spp and *Moorea* spp. Additionally, the whole genome of isolate CS953 had a high similarity to the sequenced genome of *T. erythraeum*, suggesting that it represents a benthic ecotype of *T. erythraeum*. This is despite the fact that morphologically CS953 is distinct from *T. erythraeum* and *Hydrocoleum* spp., appearing most similar to species classically identified as *Lyngbya.* Further bioinformatic analysis investigated the genome of CS953 for the presence of gene clusters for known toxins and *nif*HDK genes, responsible for nitrogen fixation. No known toxin genes were identified however the analysis suggested putative unknown toxins. The presence of the *nif*H gene confirms that this isolate is diazotrophic, a trait known in *T. erythraeum* but not found in the recently described *Moorea* spp. This study shows distinct differences in the taxonomy and toxin genes of benthic cyanobacteria from Moreton Bay, further research is needed to fully elucidate this species.

S5.4

Phylogeography of the invasive freshwater Raphidophyte *Gonyostomum semen*

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Gonyostmum semen is a freshwater raphidophyte that is currently considered invasive in Northern Europe. *Gonyostomum semen* can form dense blooms and can represent up to 95% of the phytoplankton community. This species is considered harmful as it expulses mucous threads leading to skin irritation and can clog filters used for the production of drinking water. *Gonyostomum semen* has increased in occurrence and abundance in the Northern European countries since the 1980s, and more recently in Northern Eastern Europe. Here, we aimed at studying the phylogeography of *G. semen* using strains from Northern Europe, USA and Japan. Three ribosomal DNA regions (SSU, ITS and LSU) and one mitochondrial DNA marker (cox1) were analysed. The SSU and partial LSU sequences were identical. In contrast, the mitochondrial marker cox1 showed clear differences between the Northern European and the Japanese and American strains. However, only a single haplotype was detected in Northern Europe for the cox1 region. The ITS region was cloned to determine sequence variations within strains and to identify sequences variability between strains. The overall low diversity and weak geographic structure detected in the study supported the hypothesis of a recent invasion of new lakes by *G. semen* in Northern Europe.

S5.5

Global patterns of pelagic dinoflagellate diversity across protist size classes

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Dinoflagellates (Alveolata) are one of the ecologically most important groups of modern phytoplankton. Their complexity makes assessment of their global diversity and community structure difficult. Here we used the metabarcoding approach to characterise >170 million V9 18S ribosomal DNA sequences from 106 size-fractionated plankton communities collected across the world's surface oceans during the Tara-Oceans expedition (2009-2012) in order to assess patterns of pelagic dinoflagellate diversity, toxic species biogeography and community structuring over global taxonomic and ecological scales. Our data suggest that dinoflagellate diversity has been largely underestimated, representing overall about half of total known protistan rDNA OUT (Operational Taxonomic Unit) richness in the world's surface oceans. Dinoflagellate OTU richness and abundance display at both time and spatial scales regular and differential patterns of taxonomic composition at the order level across size fractions. While the pico- and nano-planktonic communities are composed of an extreme diversity of OTUs assigned to Gymnodiniales or simply undetermined, most micro-dinoflagellate OTUs relate to the well-referenced Gonyaulacales and Peridiniales, and a lower abundance and diversity of essentially symbiotic Peridiniales is unveiled in the meso-plankton. Interestingly toxic genera (*Alexandrium*, *Dinophysis*) are present in off-shore, oceanic regions where their reports are rare. Our analyses show the power of metabarcoding to semi-quantitatively assess biodiversity from a compartment of plankton ecosystems that still escape microscopy investigations, and illustrate its promises to understand and model the dynamics and function of pelagic biodiversity.

S6.1

Death from below: disinfection by-products counteract potential eutrophication-driven harmful algal blooms in the Southern California Bight

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Blooms of harmful and toxic algae have increased in frequency and severity with coastal California, an area representing approximately 1,000 miles of the Pacific coastline of the U.S. Although several HAB organisms are present, *Pseudo-nitzschia* (Domoic Acid Poisoning) is rapidly becoming the single greatest threat and problem for human and ecosystem health, potentially eclipsing Paralytic Shellfish Poisoning in severity due to the diverse impacts on the economy through commercial fisheries and tourism, as well as via direct impacts on marine birds and mammals. As part of a multi-year, interdisciplinary program we have been identifying ecophysiological conditions leading to bloom and toxin initiation for *Pseudo-nitzschia*, by simultaneously comparing two "hot spots", Monterey Bay and San Pedro, California. Monterey Bay is dominated by coastal upwelling, while as much as 50% of the nitrogen fueling blooms in Southern California originates with regional Publicly Owned Treatment Works (POTW). During 2010 we had an unprecedented opportunity to test the hypothesis that POTW discharge of nutrients into the shallow coastal ocean would stimulate harmful algal blooms. Contrary to expectations, no bloom occurred, even though subsequent experiments in the same region demonstrate *Pseudo-nitzschia* and other algae respond strongly to nitrogen additions. We present evidence that the POTW discharge created a toxic plume dominated by disinfection by-products, highlighting the potential impacts of both coastal eutrophication and the unintended consequences of other discharge byproducts.

S6.2

Multiple stressors on the potential toxicity of *Heterosigma akashiwo* under future ocean conditions

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As the most predominant fish-killing flagellate in the Salish Sea (Pacific Northwest of North America) *Heterosigma akashiwo* has been responsible for the death of penned and wild fish stocks. Blooms of this species have been recognized for the last 20 years and occur sporadically based on presently undefined environmental conditions. The Salish Sea is strongly influenced by a diversity of ocean water intrusions and by direct and indirect human activities. The predicted alteration in the environment is the combination of ocean acidity and nutrient enrichment. Here we present laboratory findings on multiple stressors on the fish-killing flagellate *Heterosigma akashiwo*. Using future ocean projections as a model we examined growth and toxicity on the combination of increased ocean acidification (pH), altered salinities, increased temperature and modified nutrient supply. A fish gill cell bioassay was used to quantify the ecotoxicological response, associated with the fish-killing potential. The toxicity and growth responses of batch cultures, conducted at a range of initial concentrations of nitrate and phosphate (N:P ratios from 1:1 to 48:1) and 3 pH levels were considered ((pH 8.1, pH 7.4 and pH 7.8). This study demonstrates that *H. akashiwo* experienced maximal growth rates at 20 psu, which increased with increasing temperatures predicted in a global climate change scenario (from 14.7 °C to 24.4 °C). The growth rates and toxicity of *H. akashiwo* cells are not going to be affected by ocean acidification, but there were alterations in the levels of toxicity under different growth stages.

S6.3

Effects of the El Niño-Southern Oscillation (ENSO) teleconnections on the abundance of micro-phytoplankton and *Alexandrium catenella*, in Southern Chile

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An increase of the frequency and intensity of harmful algal blooms associated to Paralytic Shellfish Poison (PSP) have occurred in Southern Chile. During the seventies and eighties, *Alexandrium catenella* and PSP outbreaks in the Magellan region (48º – 55º S) appeared every 8-9 years, but since 1991 they are continuously present. PSP outbreaks and *A. catenella* variations in its abundances since 1972 are showing that these events have occurred at the end of the La Niña events but prior to an intense or medium intensity El Niño. For the Magellan region, micro-phytoplankton abundance and relative abundance of *A. catenella* (RA) time series, since 1994 are available. Through non parametric correlations, the standardized anomalies of these variables were contrasted with pluviometry and air temperature standardized anomalies, as well as with the Multivariate ENSO Index (MEI) and the Southern Oscillation Index (SOI). Our findings are showing that both biotic variables exhibit a significant correlation with MEI, pluviometry and air temperature standardized anomalies, but SOI does not always show a significant relationship. And particularly for 1997-1998, corresponding to the second most intense El Niño phenomenon of the last century, a striking increase of phytoplankton abundance and RA occurs. It is suggested that micro-phytoplankton abundance, RA and abundances of another dinoflagellates are linked through teleconnections to the ENSO, showing changes in rain fall pattern, air temperature and probably glaciers melting, and suggesting a macro-scale triggering factor regulating the micro-phytoplankton and *A. catenella* distributions and abundances, but later would be modulated by local factors.

S6.4

The raise of harmful algal blooms in Abu Dhabi coastal waters-The potential role of eutrophication and climate change

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Increasing frequency of harmful algal blooms (HAB) and subsequent environmental catastrophes are most compelling and raising threat for the Arabian Gulf's coastal resources, economy and public health. Eutrophication and climate changes are the two processes that may promote the harmful algal blooms in Abu Dhabi waters. To understand the role of these two processes, an intensive marine water quality monitoring program was initiated in 2001 and data from the past 12 years reveal that the harmful algal bloom incidents are significantly increasing every year. The changing patterns of phytoplankton taxa and the frequency of bloom formation are related to eutrophication and regional climate change, in particular, changes in temperature and salinity. Surges in human activity and coastal development driven by economic growth over the past 12 years have resulted in increased nutrient discharges, thus intensifying eutrophication in Abu Dhabi's waters. The nutrient levels have increased many folds than the normal sea water and the blooms produced were associated with widespread harmful impacts including hypoxic events (0.20mg/L), finfish kills (*Nematalosa nasus*) and loss of bottom living organisms. Extensive dredging and land reclamation activities could also have exacerbated the situation and contributed towards eutrophication and subsequent alteration of the ecosystem. Since Abu Dhabi is experiencing extreme climatic condition, it is highly vulnerable to the effects of regional climate change. Apart from the eutrophication, the role of climate change in phytoplankton genera and harmful algal bloom expansion will be discussed in detail. In addition, the paper will also focus on the details of efforts made by the Environment Agency-Abu Dhabi on HAB prevention, control and mitigation to protect the environment and public health.

S6.5

Cyst distribution, viability, and growth characteristics of *Alexandrium tamarense* (Dinophyceae) from Arctic waters

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Observational data show that the Arctic Ocean is undergoing significant and rapid climate-related changes. These changes are expected to impact the geographic range of a variety of marine taxa, including toxin-producing phytoplankton responsible for harmful algal blooms. Recent studies have documented high densities of *Alexandrium tamarense* dinoflagellate cysts in the Chukchi Sea, as well as low concentrations of vegetative cells and associated phycotoxins in the coastal waters of western Greenland; however, their origin and prevalence are not established. To better understand the distribution and physiology of *A. tamarense* in Arctic waters, we examined sediment samples collected during the ARCHEMHAB research expedition in western Greenland and Iceland for *Alexandrium* cyst abundance. Additionally, we examined the growth characteristics and toxin content of *A. tamarense* cultures established from these samples, and from samples collected in the Chukchi Sea. Analysis of sediments showed that *Alexandrium* cysts were present at low to moderate densities in most areas surveyed in Greenland and Iceland, with highest densities observed in western Iceland. Preliminary data from our physiological studies suggest that the growth characteristics of Arctic isolates in response to temperature are similar to that of temperate isolates; however, modest growth observed at low temperatures may be sufficient for bloom development and maintenance in Arctic waters. Data from these studies help to assess the current distribution and processes promoting the expansion of toxin-producing phytoplankton in the Arctic, and will aid in the identification of dispersal pathways, and of regions and seafood resources that are at risk.

S7.1

'Toxic in Crowds' – Inside a *Microcystis* scum; profiling microcystins, pH, dissolved oxygen and photosynthetic activity

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Blooms of microcystin-producing *Microcystis* are problematic worldwide and contact with contaminated water has resulted in multiple human and animal fatalities. The mechanisms that regulate toxin production are still highly debated. Previous work by our research group has shown that the formation of *Microcystis* scums leads to an increase in microcystin quota (up to ca. 20-fold). In order to improve knowledge on whether up-regulation is caused by increased cell density, or by the 'stressful' i.e., elevated pH and dissolved oxygen, conditions inside a scum, we added concentrated suspensions of *Microcystis* to an on-land mesocosm. Over 24 hours (as the scum formed from the mixed suspension) the pH and dissolved oxygen of the surface 2-3 mm were profiled in 100 µm steps using a benthic lander equipped micro-electrode probes. Monitoring pulse amplitude modulation fluorometers were positioned above and below the scum to assess photosynthetic efficiency. During the study, samples were collected for determination of microcystin (liquid chromatography-mass spectrometry), dissolved inorganic carbon, nutrients, microscopic examination and microcystin synthase gene expression. New cryo-sampling techniques were tested to enable precise surface and profile samples from the cyanobacterial scum to be collected. These data are being used to elucidate diurnal shifts within *Microcystis* scums and to examine the environmental conditions which cells are exposed to. The results of this study could provide valuable insight into the environmental triggers of microcystin production.

S7.2

'Toxic in Crowds' – Using small scale mesocosms and modelling approaches to explore regulation of microcystin production

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Microcystins are the most commonly detected freshwater cyanobacterial toxin worldwide and are usually associated with the bloom forming cyanobacteria genus *Microcystis*. Despite the global occurrence of toxic *Microcystis* blooms, the parameters that regulate microcystin production remain uncertain. Laboratory culture-based studies have shown correlations between microcystin production and a range of physiochemical variables (e.g., nutrients and temperature). However, results are often ambiguous and usually induce only 3- to 4-fold changes in microcystin production. Recent field-based studies by our research group manipulating *Microcystis* cell densities in mesocosms resulted in an increase of ca. 20-fold in intracellular microcystin over a 5 hour period. Further in-depth studies are required to establish whether this is related to cell density or other bloom-related variables that can be mutually correlated with cell density. In this study four separate mesocosm experiments were undertaken investigating the effect of pH, light vs. dark, nitrogen and cell density. Experiments were undertaken in 55 L mesocosm and lasted between 6 to 22 hours. Samples were collected for microcystin, dissolved inorganic carbon, nutrients, microscopy, molecular analysis (DNA/RNA) and a suite of physical parameters were measured *in-situ*. These data are being used in concert with a one-dimensional hydrodynamic model (DYRESM) to investigate how vertically-based mixing and changes in water column light penetration and stratification interact within individual mesocosms and effect cell density and microcystin production. Our ultimate goal is to use a three-dimensional model (ELCOM-CAEDYM) to extrapolate how *Microcystis* densities and environmental factors influence microcystin production at a whole lake scale.

S7.3

Impact of connectivity and distance on population divergence of an invasive nuisance microalgae

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The nuisance microalgae *Gonyostomum semen* has successfully spread within 50 years from a few lakes in Southern Scandinavia to the Arctic Circle. Nevertheless, an earlier study reported first signs of differentiation of this species into genetically distinct populations. Genetic divergence despite high spreading capacity indicates significant physical and/or biological dispersal barriers, which prevent gene flow between populations of different lakes. In this study we sampled *G. semen* strains from 6 lakes in two watersheds, to examine the importance of connectivity and distance for the dispersal and divergence of microalgae populations. We genotyped the individual isolates by Amplified Fragment Length Polymorphism. F-statistics showed slight but significant differentiation between the sample locations, and the null hypothesis of panmixia was refuted. Analysis of molecular variance showed that connectivity and watersheds could only explain a minor part of the observed genetic differentiation of populations. We suggest that biological dispersal barriers like local adaptation and founder effects are more important for population divergence in microalgae than connectivity and isolation by distance.

S7.4

First detection of microcystin in Puget Sound, Washington mussels: Ecological and human health implications

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Direct exposure or consumption of water with cyanobacteria toxins, such as microcystin (MC), present known health risks to humans. However, other exposure pathways, including consumption of contaminated "seafoods" is much less understood. Studies have documented MC accumulation in seafoods from freshwater environments, but monitoring for MC presence in marine ecosystems has been minimal despite confirmation of MC contaminated freshwater entering these waters. In the Pacific Northwest region of the United States, numerous freshwater lakes that typically are subject to summer cyanobacteria blooms, drain into Puget Sound, an inlet of the Pacific Ocean. These freshwater drainages are potential sources for MC contamination of fish and shellfish growing in the Puget Sound receiving waters. Puget Sound shellfish are important economic resources that are commercially distributed worldwide and are harvested by many residents as a locally available, inexpensive protein source. A pilot study was developed to determine if saltwater mussels, *Mytilus trossulus*, growing in Puget Sound accumulate MCs. MC was detected, by liquid chromatography-tandem mass spectrometry, in all but one Puget Sound mussel exposed to discharge from a lake with maximum concentrations of 52.4 ppb. This is the first known report of marine bivalves in the northwestern United States accumulating MC from freshwater sources. Our results suggest that MCs originating in freshwaters may be transferred through Puget Sound food webs. The ecology of cyanotoxin movement in aquatic food webs and quantification of potential for human exposure through consumption of seafoods warrants greater research attention.

S8.1

A review: The distribution and damage of toxic microalgae in China coast, and ecological risk assessment

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The harmful algal blooms have occurred more frequently in the worldwide, the toxic microalgae can produce phycotoxins, which can accumulate in shellfishes by bioconcentration, and poisoning human and other high grade marine animals, which cause great loss and damage to human health, aquaculture industry, and ecological environment. Various toxic microalgae which can produce a variety of phycotoxins, are widely distributed in the sea waters of China. There are at least 4 lines of *Alexandrium spp.*, producing paralytic shellfish poisoning(PSP), in China coast from south to north,also there are 5 lines of *Dinophysis spp*. which can produce diarrhetic shellfish poisoning(DSP), okadaic acid(OA) and dinophysistoxin(DTXs) and pectenotoxins(PTXs), along the China Coast. Three dinoflagellates which can produce yessotoxins(YTXs) exists throughout in north of the Yellow Sea and Jiaozhou Bay of China. PSP, OA and DTXs, PTXs, YTXs and GYM(gymnodimine) have been found in a variety of Chinese shellfishes. This paper systematically elaborated and summarized the present situation, including the types, distribution, and harm of toxic microalgae in China Coast, and the characteristics and regularities of phycotoxins in bivalve and other marine organisms in China; and evaluated the possible health and ecological risk, as well as provided some suggestions of environmental safety and seafood safety and management.

S8.2

Red tides in a semi-enclosed embayment system, Shiwha Bay, Korea: a huge dike and tidal power plant was constructed

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To investigate red tides in Shiwha Bay, Korea, where a 12.7-km dike with two outlets (at the water gate and tidal power plant) was constructed, we measured physical, chemical, and biological properties at 3 fixed stations inside the dike on a monthly basis from May 2008 to July 2012. During the study period, red tides were present in Shiwha Bay during 33 of 46 (i.e., 72%) sampling events. Clearly, red tides are common in the bay. Red tides occurred 33, 12, and 10 times at Stations 1, 2, and 3, respectively. Restriction of water circulation at Station 1 may cause more frequent red tides due to phototrophic dinoflagellates than at Station 3, where water exchange between the inside and outside of the dike occurs through a water gate. After the world's largest tidal power plant was established in 2011, red-tide causative species switched from phototrophic dinoflagellates to diatoms. Exchange of seawater between the inside and outside of the dike through the tidal power plant may have resulted in this change in the causative species. Inorganic nitrogen concentrations for the growth of phototrophic dinoflagellates and small flagellates during red tides were likely unlimited, but inorganic phosphorus concentrations may be limited. Thus, some phototrophic dinoflagellates and flagellates may acquire phosphorus from prey. The maximum grazing coefficients of the heterotrophic dinoflagellates feeding on red-tide causative taxa were found to be 0.14–0.77 h^{-1}. Therefore, heterotrophic protistan grazers in Shiwha Bay may, at times, have considerable grazing impact on populations of co-occurring red-tide organisms.

S8.3

An unusual persistence of toxic *Dinophysis* species in the Gulf of Trieste (Adriatic Sea) caused economic loss to local mussel farmers

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According to phytoplankton species present in the Gulf of Trieste (Adriatic Sea), consumers of the local farmed mussels are potentially exposed to three types of intoxication problems. Besides amnesic (ASP) and paralytic shellfish poisoning (PSP), of which the causing toxins were never found to exceed the legal limits in mussels farmed in the area, the only toxins that are regularly detected are lipophilic toxins, mainly those responsible for diarrhetic shellfish poisoning (DSP). During monitoring of toxic phytoplankton, an unusually long persistence of some *Dinophysis* species (mainly *D. caudata* and *D. fortii*) in the sea water was recorded in the year 2010. Cells were detected in the samples from May to November and reached abundances up to 2000 cells/L, which is far more than regularly found abundances of a few hundred cells/L. Enhanced water column stratification driven by a low salinity surface layer (salinity range 30-35) was observed concomitantly to *Dinophysis* presence. Toxin identification in phytoplankton extracts with the use of LC-MS/MS revealed presence of OA and PTX2. Positive results of mouse bioassays performed in the frame of mussel toxicity monitoring program led to a particularly long lasting closure of shellfish harvesting from June 2010 to April 2011. This toxicity event was presumably the result of atypical environmental characteristics and, in combination with methodological problems due to mouse bioassay low selectivity and specificity, caused a significant loss of income to local mussel farmers (about 800,000 €). The bioassay was eventually replaced by the spectrophotometric method only in July 2011.

S8.4

Occurrence, danger and control of harmful algal bloom in ponds for marine aquaculture

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Along the Liaodong Bay, northeastern China, harmful algal bloom frequently occurred due to eutrophication. If harmful algal bloom enters ponds, it will pose a serious threat to aquaculture. In recent years, we conducted a series of studies involved in occurrence and danger of the bloom of *Gymnodinium gracilentum*, *Exuviaella*sp.and *Prymnesium parum*in many ponds for aquaculture of *Litopenaeus vannamei*, *Rhopilema esculenta*, *Eriocheir sinensis* and rotifer. Furthermore, we used biological manipulation to control the harmful algal bloom. For example, we successfully used *Chlorella* to control the bloom of *Gymnodinium gracilentum, Brachionus plicatilis*to control *Amphidinium* sp. and *Gymnodinium* spp., ammonium salt to control *Amphidinium* sp., and used *Artemia salina*to control *Exuviaella minima* bloom. These methods have successfully been extended to large-scaled aquaculture, which indicated that biological manipulation was effective to control the occurrence of harmful algal bloom in marine aquaculture in ponds. We also summarized the methods of using clay grout to reduce the danger of harmful algal bloom in aquaculture.

S10.1

Diversity and seasonal dynamics of small dinoflagellates in the Skagerrak, Norway revealed by 454 pyrosequencing and microscopy

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Some dinoflagellates are small and difficult to identify under the light microscope and thus little is known about their seasonal dynamics at the species level. Here we combined high-throughput sequencing and microscopy to explore their diversity and seasonal dynamics, and relate this to environmental factors. We collected 21 monthly samples of nanoplankton (3-45 µm size fraction) from outer Oslofjorden, southern Norway over two years. The 18S V4 ribosomal DNA was amplified from RNA/cDNA and 454-pyrosequenced. The reads were cleaned, denoised and clustered to 99% in Qiime and assigned to taxon. From 46250 reads assigned to Dinoflagellata we detected 367 unique operational taxonomic units (OTUs) assessed to represent species or genera. The most OTU-rich orders were Gymnodiniales (32%), Syndiniales (22%), and Peridiniales (17%), followed by Dinophysiales (8%), Gonyaulacales (7%) and Suessiales (4%). Reads assigned to Gymnodiniales dominated in all samples except June and August 2010 when reads assigned to Suessiales dominated. We observed strong seasonal variation and significant yearly periodicity in dinoflagellate richness and species composition. OTU richness was highest in late autumn and lowest in winter-spring. Seasonal dynamics of potentially toxic taxa were examined. We also assess and discuss the ability to detect potentially toxic dinoflagellate species with 18S V4 high throughput sequencing and compare this with standard microscopical methods and electron microscopy. Potentially toxic species <45 µm observed under the microscope included e.g. *Alexandrium pseudogonyaulax, A. ostenfeldii, Azadinium spinosum, Karenia mikimotoi, Karlodinium veneficum* and *Prorocentrum minimum.*

S10.2

Alexandrium tamarense Group I as the cause of PST on the east coast of Tasmania, Australia

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In October 2012 paralytic shellfish toxins (PST) were detected in shellfish on Tasmania's east coast resulting in widespread harvest closures of mussels, oysters, scallops, rock lobster and abalone over a period of six months along 350 km of coastline with total economic losses estimated at $23M. Phytoplankton sampling indicated *Alexandrium tamarense* as the likely source, a species not previously linked to PST in Australia because past recorded blooms were low-toxicity *A. tamarense* Group 5. More than 20 cultures were established from inshore and the continental shelf-edge locations. DNA sequence analysis (LSU-rDNA, rDNA-ITS, STX synthetase SxtA1/A4) confirmed all isolates as toxic *A. tamarense* Group I, not previously known from Australasia. HPLC fluorescence analysis of PST showed a consistent profile dominated by C1/2 and GTX1/4, low proportions of NEO, C3/4, and traces of GTX2/3 and dcGTX2/3, with an 8-fold variation in STX content (8-65 fmol cell^{-1}) among strains. Sequences of *A. catenella* Group IV were also recovered from seawater and shellfish samples indicating that multiple toxic *Alexandrium* genotypes may have contributed to the 2012 event. Cells of *A. tamarense* were detected again in early July 2013 resulting in widespread PST from Flinders Island (Bass Strait) and south to the Tasman Peninsula during winter-spring. The presence of *G. catenatum* and now at least three *A. tamarense* genotypes extend PST risk in Tasmania to year-round, presenting a major challenge for Tasmanian seafood assurance and HAB monitoring.

S10.3

Diversity and seasonality of *Pseudo-nitzschia* (Bacillariophyceae) through the molecular looking glass

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Within the genus *Pseudo-nitzschia*, several genetically distinct species have been described within the same morpho-species, including several toxic species. We analysed phytoplankton samples and clone libraries obtained from environmental DNA amplified with a genus-specific pair of 28s rDNA (LSU) primers to study the diversity and seasonal patterns of taxa within the genus *Pseudo-nitzschia* in the Gulf of Naples (Mediterranean Sea). To this end, 19 seawater samples were collected in surface waters at the station LTER-MC over 16 months in 2009 and 2010. Based on 1643 useful sequences, we identified 17 *Pseudo-nitzschia* species, of which two were new for the area, and at least two other undescribed ribotypes. Several species showed a high level of genetic diversity, while others were lowly diverse, indicating different rates of diversification among closely related taxa. Different cryptic species within the same morpho-species showed distinct temporal patterns of occurrence, with the vast majority of these species being restricted to seasonal periods of variable length and only a few being detected virtually throughout the seasonal cycle. A good match was found between microscopic and molecular results at least for the seasonal distribution of species recognisable in LM, while clone libraries tended to overestimate the relative abundance of some of the species. Due to its high resolution and detection power, the metabarcoding approach used reveals to be an optimal tool to trace the distribution of cryptic and toxigenic *Pseudo-nitzschia* species in the natural environment and to increase the knowledge of their diversity.

S11.1

Morphological and molecular characterization of eukaryotic parasitoids on toxic marine dinoflagellate *Alexandrium tamarense*

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The diversity and ecological roles of eukaryotic parasitoids in marine environment are poorly known. Here we report the presence of parasitoids that were found during the germination experiments of the cysts of *Alexandrium tamarense.* Sediment suspensions prepared with the bottom sediment from Ofunato Bay, northern Japan, were incubated at 12.5˚C in the light. During the incubation, the germinated cells with abnormal chlorophyll autofluorescence or the cysts of aberrant morphology were isolated and incubated individually for subsequent microscopic observations. Fully matured sporangia were isolated for the rDNA sequence analysis. Two distinct parasitoids (Sp.1 and Sp.2) were found. Sp.1 and Sp.2 infected the germinated cells and the resting cysts, respectively. The parasitoids in the host cytoplasm developed, matured and produced numerous zoospores. The zoospores were liberated through discharge slits (germ tubes) in Sp.1 and through prominent papillae in Sp.2. The zoospore of Sp.1 was peanut-form of ca. 4 µm long by 2 µm wide and had two flagella. The zoospore of Sp.2 was spherical of ca. 3µm in diameter and had tentacle-like processes, together with two flagella. On the basis of 18S rDNA sequence analysis Sp.1 thought to be a species belonging to *Parvilucifera*. Although rDNA sequence revealed that Sp.2 belong to 'chytrids' *sensu lato*, its taxonomic position was not clarified. Our finding indicates that a variety of parasitoids can have significant impacts on *Alexandrium* and parasitism needs to be considered in understanding of the population dynamics. In addition, these parasitoids might to be potential agents in the control of the blooms.

S11.2

Occurrence of *Amoebophrya* spp. infection in planktonic dinoflagellates in coastal waters of China

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The parasitic dinoflagellates in the genus of *Amoebophrya* can infect broad ranges of planktonic dinoflagellates, and transform algal biomass into organic matter that can be recycled within the planktonic community. The ecological significance of *Amoebophrya* spp. during harmful algal bloom (HAB) events was gradually recognized along with revelation of its host specificity and diversity. The eutrophicated coastal waters of China are frequently affected by HABs; while, no research has been conducted to explore the ecological roles of parasitism during HAB events. Thus, to investigate the presence of *Amoebophrya* spp. in planktonic dinoflagellates, we collected environmental water sample from sporadic sites in the Bohai Sea and the Yellow Sea, and intensive sites at Changjiang (Yangtze River) estuary and the adjacent East China Sea (ECS). For the first time, we confirmed the presence of *Amoebophrya* infections in the planktonic community from coastal waters of China; eight species of dinoflagellates were infected, including *Ceratium tripos*, *Scrippsiella trochoidea*, *Gonyaulax spinifera*, *Prorocentrum minimum, Katodinium glaucum, Gymnodinium* sp., *Gonyaulax* sp. and an *Alexandrium* sp. Molecular sequences retrieved from environmental water samples revealed high genetic diversity of Amoebophryidae-like organisms in the water column. In Changjiang estuary and the adjacent ECS, *Amoebophrya*-infected dinoflagellates were only observed in high salinity (>20) stations, suggesting that salinity may be a factor limiting the distribution of *Amoebophyra* infections in natural environment. Currently, *Amoebophrya* infections were mainly identified in no-dominant species of dinoflagellates, whether *Amoebophrya* infection can significantly affect the dynamics of typical bloom-forming dinoflagellates still needs to be further investigated.

S11.3

The parasitoid *Parvilucifera sinerae* that kills toxic marine dinoflagellates: An overview from cell to field population

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Parvilucifera sinerae is a parasitoid that infects dinoflagellates, including harmful algal bloom (HAB) species. Here we describe the *P. sinerae* life cycle, in which a free-living zoospore penetrates a host cell and then develops a trophocyte while destroying the host cytoplasm. The trophocyte undergoes schizogony to form hundreds of new zoospores inside a sporangium. This sporangium remains dormant until, in response to an activation signal, the zoospores are released into the marine environment, where they are able to infect a new host. We identified dimethylsulfide (DMS) as the chemical signal involved in zoospore activation. Since many marine microalgal species are DMS producers, *P. sinerae* may be activated by many species. This fact is consistent with the results obtained in a previous study, where we found that *P. sinerae* is a generalist parasitoid, based on its broad host range among dinoflagellate species. Laboratory experiments demonstrated a prevalence of *P. sinerae* over 80% of the dinoflagellate population, causing the complete extermination of the host population after two parasitoid generations. The exploitation of dinoflagellate parasites in HAB mitigation has been proposed by several authors. However, as in many parasitic systems, the dynamics of *P. sinerae* infection are host-density-dependent, such that laboratory experiments poorly reproduce host-parasite interactions in nature, and field studies are lacking. In this work, we also present preliminary results of the prevalence of *P. sinerae* during a winter bloom of *A. minutum* in Arenys de Mar harbor (NW Mediterranean Sea) and discuss the parasite's potential contribution to bloom termination.

S12.1

Improvement of harmful algal bloom models through advanced data assimilation and adaptive modeling

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Even though much progress has been made recently in modeling harmful algal blooms, uncertainties remain much larger than in ocean circulation models. Fundamental equations governing the dynamics of phytoplankton species and their interactions remain relatively unknown. As a consequence, many formulations in harmful algal bloom models are based on empirical relationships, which imply an excessive degree of parameterization and restrictive assumptions. Here, we present adaptive modeling as an extension of data assimilation to the selection of model state variables, linkages, parameterizations and mathematical formulations. The regular model state variables are combined with model formulations, forcing fields and parameters in an augmented state vector, which is updated through an ensemble Kalman filter. Based on misfits between model predictions and real data, adaptive modeling identifies model structures and formulations that need to be improved, estimates those improvements, and corrects the model accordingly. The model changes and learns from data providing more realistic predictions and selecting the most adequate model formulations that describe the system. We use this adaptive modeling approach to identify the factors (e.g. life cycle strategy, nutrient availability, species competition, or zooplankton grazing) that determine *Alexandrium minutum* blooms in Arenys de Mar harbor (northwestern Mediterranean Sea).

S12.2

Towards an operational HAB forecasting system for coastal California

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A feasibility demonstration is underway for operational HAB forecasting in coastal California, building on years of proof-of-concept studies in various hot spots off the U.S. West Coast. This project introduces a method for predicting the spatial likelihood of *Pseudo-nitzschia* blooms and dangerous levels of domoic acid (DA) using a unique blend of numerical models, ecological forecast models of target phytoplankton species, and satellite ocean color imagery. What we consider to be the most innovative aspect of our approach is the merger of satellite data with numerical forecasts of the physical data to statistically reconstruct biogeochemical fields up to three days to then force our existing statistical models for forecasting HAB events. Daily predictions that merge reconstructed satellite fields with ROMS model output are run routinely at the Central and Northern California Ocean Observing System (CeNCOOS) and posted on their website for dissemination to a select group of test end-users in aquaculture, public health, and marine mammal management. We present a case study for Spring 2014, one of the largest domoic acid events on record with unprecedented closures of sardine and anchovy fisheries in the Monterey Bay region. Model predictions were well correlated at a week lead-time with shellfish and fishery closures. Historical stranding data are compared with model hindcast runs, and marine mammals appear to be good sentinels of the offshore onset of a DA event at large spatial scales. Citizen observations of mammal strandings as part of this project provide quality control for offshore DA predictions.

S12.3

Alexandrium catenella: forecasting its impact in an important coastal marine farming area

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The emerging issue of saxitoxins associated with *Alexandrium catenella* in a major aquaculture region of New Zealand has led researchers to investigate tools that may help forecast and communicate the risk of major events. Over the last five years a variety of conditions have been observed, with some years producing very high concentrations of cells leading to a closure of an important shellfish growing region. An overview of biophysical observations will be presented, accompanied by preliminary model simulations undertaken for a small embayment commonly associated with large aggregations of *Alexandrium catenella*. These findings and models will form the basis of future forecasting tool development, which will aim to assist managers in preparing for future events.

S13.1

Molecular insights in the biology of the planktonic diatom *Pseudo-nitzschia multistriata*

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In the diatom genus *Pseudo-nitzschia*, at least 12 species are known to be responsible for the production of the toxin Domoic Acid (DA). Among these, *Pseudo-nitzschia multistriata* has been reported to produce DA in moderate amounts. Ecology, life cycle and population genetics have been studied extensively in this species. We are now developing molecular tools to characterize the molecular mechanisms that underlie many of its biological features. As the species is heterothallic, it is possible to control genetics, and therefore, in principle, it is possible to use *P. multistriata* as a genetic system, setting the basis for the study of gene function. We have produced a reference genome and a reference transcriptome using Illumina sequencing, and have begun explorations of these data sets focusing on meiotic genes and on specific metabolic pathways. In addition, we have succeeded in transforming *Pseudo-nitzschia* species with the biolistic method, and a range of functional genomics approaches (overexpression, RNAi) are being explored. These tools are allowing a number of novel studies in *P. multistriata*, including the definition of signaling molecules and pathways employed for chemical communication among cells, and the exploration of the molecular signatures characterizing highly toxic strains versus non-toxic strains. The latest findings will be presented.

S13.2

High-throughput DNA sequencing reveals the distribution of harmful algal bloom species in the Baltic Sea

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To analyse phytoplankton samples to identify and quantify harmful algal bloom species is a time consuming process that relies on the skill and knowledge of the microscopist. More cost efficient methods are therefore sought after. One option is DNA sequencing based methods. Here we studied the distribution of harmful algal species in the Baltic Sea using a combination of microscopy and high-throughput DNA sequencing in summer 2013. A cargo vessel equipped with a FerryBox-system was used as a sampling platform to collect water samples from approximately 3 m depth from the Bothnian Bay to the Kattegat. Simultaneously several parameters was measured automatically, e.g. temperature, salinity and fluorescence from chlorophyll and phycocyanin. Water samples were analysed microscopically using the Utermöhl method and by Illumina DNA sequencing approximately 450 and 600 base pair long variable regions of 16S and 18S rDNA for prokaryotes and eukaryotes, respectively. Approximately 100,000 sequences were analysed from each water sample. Altogether 4146 Operational Taxonomic Units (OTUs) with >97% similarity rDNA-sequences were compared with published sequences from HAB species. Results from microscopy and rDNA show a high similarity in spatial distribution of e.g. the toxin-producing cyanobacteria *Nodularia spumigena*. Among the dinoflagellates the rDNA data show a wider distribution of *Alexandrium* spp. compared to results from microscopy. Also *Dinophysis* spp. was detected, however the rDNA sequences did not discriminate well between different species within this genus. Moreover, the high throughput sequencing of rDNA revealed the distribution of some HAB species in the Baltic Sea not detectable using regular microscopy.

S13.3

Characterisation of HAB species from the Greenland/Iceland Arctic region

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It is predicted that climate change will have dramatic consequences for the Arctic region, including increased mean temperature and rapid decline of ice cover. These ecological alterations may have the potential to expand the distributional ranges of HAB-species into or within the Arctic sea-area. As a baseline study on the presence of HAB species in the Greenland/Iceland area, a research cruise with "Maria S. Merian" was conducted in August 2012. Plankton net tow and bottle samples were qualitatively inspected for the presence of HAB species and were used for single cell isolation and subsequent cultivation. This strategy yielded 22 isolates of *Alexandrium tamarense*, 36 isolates of *A. ostenfeldii* and one of *A. tamutum*, all of which were characterized in terms of phylogeny, morphology, toxin profile and lytic activity against a protistan target. Furthermore, six isolates of *Protoceratium reticulatum* were obtained and studied in terms of phylogeny, growth, yessotoxins, and lytic activity. Finally, seven isolates representing five species of the potentially azaspiracid (AZA)-producing Amphidomataceae were established. Three new species of *Azadinium* were described from among these isolates. None of these contained known AZAs; this was also the case for *Azadinium dexteroporum*, in contrast to the literature report of AZA in a Mediterranean strain of this species. AZA production was confirmed for the Iceland isolate of *Amphidoma languida*. Our preliminary studies demonstrate the widespread occurrence of toxigenic species in the Arctic and our phenotypic and genotypic characterisations provide the basis for further investigations about the potential ecological consequences for HABs in this sensitive environment.

S13.4

Genetic structure of algal blooms: Investigating the spring bloom of the Baltic Sea in spatial and temporal dimensions

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The Baltic Sea spring bloom propagates from south to north during March to April. An important question is if the bloom consists of one genetic population that moves northward, or if different populations bloom consecutively. To study temporal and spatial connectivity of the bloom-forming diatom *Skeletonema marinoi* we conducted four cruises along a southwest-northeast transect during the spring bloom 2013. 2000 individuals were isolated at ten fixed stations, and genotyped with eight microsatellite loci. F-statistics showed significant differentiation between the stations, refuting the null-hypothesis of panmixia. Pooled individuals from all four cruises at each station displayed significant isolation by distance. Populations sampled at the same station at different times were not differentiated. A sea-scape approach investigated if oceanographic connectivity could explain patterns of gene flow. Results indicate that different basins constitute physical dispersal barriers, which retain their native populations and reduce gene flow. Spatial structure was observed within the Arkona basin despite apparent absence of dispersal barriers. We hypothesize that retention by accumulation of dormant seed populations, in combination with local adaptation is important to explain the observed genetic structure.

S13.5

A bioinformatics survey on the metagenome of the red tide dinoflagellate *Pyrodinium bahamense var. compressum*

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The dinoflagellate *Pyrodinium bahamense var. compressum* is reported to be host to a number of bacterial endosymbionts. High throughput sequencing of genomic DNA extracted from *Pyrodinium bahamense var. compressum* algal cultures from Sorsogon Bay, Philippines resulted in a metagenome mix of eukaryotic and prokaryotic sequences. Sequence reads were assembled and sorted by genomic features to group into a bacterial or dinoflagellate source and then validated by eukaryotic poly-A captured mRNA sequence data. To identify several endosymbionts present in the metagenome 16S genes were extracted from the assemblies and identified by BLAST and whole genome mapping of sequence reads were done on suspected endosymbionts present. Selected genetic traits were also examined in the host dinoflagellate: (i) assembly contigs with characteristics of a dinoflagellate mitochondrion genome were compiled, (ii) saxitoxin genes *sxtA* and *sxtG* were found and are highly similar to *Alexandrium fundyense sxtA* and *sxtG* genes, and (iii) potential splice leaders were identified. This small representative of the large dinoflagellate genome will help provide new clues for more targeted researches.

S14.1

Distribution and ecophysiology of red tides by mixotrophic dinoflagellate in Korean waters during last 30 years

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We investigated the outbreaks of red tides dominated by mixotrophic dinoflagellates in the coastal waters of Korea from 1981 to 2009. During the study period, 693 mixotrophic dinoflagellate red-tide events occurred. Of these, 36.8% were *Cochlodinium polykrikoides* red tides, with *Akashiwo sanguinea, Ceratium furca,* and *Prorocentrum minimum* also frequently forming red tides. Eighty-five percent of mixotrophic dinoflagellate red tides in Korean waters occurred from June to September, in the high water-temperature season. The monthly frequency of outbreaks of mixotrophic dinoflagellate red tides increased from twice in January – March to 228 times in August, but it decreased to 2 – 9 times in November and December. Eighty-six percent of the outbreak events were recorded in southern coastal waters, 12% in eastern coastal waters, and only 2 % in western coastal waters of Korea.

S14.2

Daily variations in heterotrophic protists in the dynamics of red tides in Masan Bay, Korea

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We measured the abundance of heterotrophic dinoflagellates, ciliates, and heterotrophic nanoflagellates in daily samples collected from June 2004 to May 2005 to investigate the role of heterotrophic protists in the dynamics of red tides in Masan Bay, Korea. The HTDs *Gyrodinium dominans/Gyrodinium moestrupii, Gyrodinium glaucum, Protoperidinium bipes,* and *Pfiesteria*-like *dinoflagellates* (PLDs) as well as naked ciliates (≤50 ㎛) were present nearly all year and their maximum biomass was 235, 48, 298, 1020, and 1013 ng C ml^{-1}, respectively. PLDs were the most abundant taxa during red tides dominated by *Akashiwo sanguinea, Heterocapsa rotundata,* summer populations of *Prorocentrum minimum, Heterosigma akashiwo, Eutreptiella gymnastica,* and cryptophytes, while *G. dominans/G. moestrupii* were most abundant during red tides dominated by *Ceratium furca* and *Dinophysis acuminata*. Naked ciliates were most abundant during red tides dominated by *Cochlodinium polykrikoides, Prorocentrum triestinum,* and winter populations of *P. minimum*. The maximum calculated grazing coefficients for each dominant heterotrophic protistan grazer on their respective co-occurring red-tide organisms in Masan Bay ranged from 0.0 to 6.8 h^{-1}.

S14.3

Feeding by heterotrophic protists on the free-living dinoflagellate S*ymbiodinium* sp.

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To investigate heterotrophic protists grazing on *Symbiodinium* sp., we tested whether the common heterotrophic dinoflagellates *Gyrodinium dominans*, *Gyrodinium moestrupii*, *Gyrodinium spirale*, *Oblea rotundata*, *Oxyrrhis marina*, and *Polykrikos kofoidii* and the ciliates *Balanion* sp. and *Parastrombidinopsis* sp. preyed on the free-living dinoflagellate *Symbiodinium* sp. (clade E). We measured the growth and ingestion rates of *O. marina* and *G. dominans* on *Symbiodinium* sp. as a function of prey concentration. Furthermore, we compared the results to those obtained for other prey species. In addition, we measured the growth and ingestion rates of other predators at single prey concentrations at which these rates of *O. marina* and *G. dominans* were saturated. All predators tested in the present study, except *Balanion* sp., preyed on *Symbiodinium* sp. The specific growth rates of *O. marina* and *G. dominans* on *Symbiodinium* sp. increased rapidly with increasing mean prey concentration < ca. 740–815 ng C/ml (7,400–8,150 cells/ml), but became saturated at higher concentrations. The maximum growth rates of *O. marina* and *G. dominans* on *Symbiodinium* sp. (0.87 and 0.61/d) were higher than those of *G. moestrupii* and *P. kofoidii* (0.11 and 0.04/d). *Symbiodinium* sp. didn't support positive growth of *G. spirale*, *O. rotundata,* and *Parastrombidinopsis* sp. However, the maximum ingestion rates of *P. kofoidii* and *Parastrombidinopsis* sp. (6.7–10.0 ng C/predator/d) were higher than those of *O. marina* and *G. dominans* on *Symbiodinium* sp. (1.9–2.1 ng C/predator/d). The results of the present study suggest that *Symbiodinium* sp. may increase or maintain the populations of some predators.

S14.4

Occurrence and dynamics of mucus trap formation among *Alexandrium* spp., with special emphasis on *Alexandrium pseudogonyaulax*

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Recently, a novel feeding strategy, the toxic mucus trap, was discovered in the dinoflagellate *Alexandrium pseudogonyaulax*. In this study, over 40 strains of 8 different *Alexandrium* species were screened for their ability to form a mucus trap and ingest prey. The mucus trap feeding strategy remains unique to *A. pseudogonyaulax.* To further realize the impact of this strategy, details of the trap were examined, such as speed and frequency of trap formation and duration and strength of the effects. The percentage of *A. pseudogonyaulax* cells producing a mucus trap and the number of prey cells caught increased with increasing prey concentration, whereas the physical size of the traps was independent of prey concentration. In one strain given an excess of prey, within 1 hour over 90% of individual *A. pseudogonyaulax* cells had formed a trap, each containing an average of 45 cells. Production of traps by an individual was nearly continuous and up to 8 traps were produced by a single *A. pseudogonyaulax* cell after only 24 hours. The attachment of an *A. pseudogonyaulax* cell to the trap only ceased during, and just following, cell division. Prey cells were, to some extent, capable of freeing themselves from the mucus trap, but it remained sticky and continued catching prey up to 48 hours after the trap had been discarded. These results reveal that the effects of the mucus trap extend far beyond the removal of prey through ingestion, and the potential impact of this strategy on surrounding cells remains high.

S14.5

Sources of dissolved organic nitrogen in catchments and implication for harmful algae blooms

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Harmful algal blooms (HABs) have been shown to use dissolved organic nitrogen (DON) when inorganic nitrogen sources are not available, with urea being the most studied form. However, the sources, transformations and fate of DON in catchments have typically received little attention. This study examined DON loads leaching from two types of vegetation, riparian and pasture areas in a subtropical agricultural catchment to determine their contribution to DON loads downstream. Soils from riparian areas leached more DON than pasture areas. The growth response of cyanobacterial species dominating in reservoirs to DON loads from catchments was also examined and will be discussed. This study has implications for efforts to reduce catchment N loads downstream by increasing riparian vegetation.

S15.1

Morpho-functional groups for Lake Geneva – A key first step to model and predict the succession of cyanobacteria under future climate change scenario

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Predicting the stochastic pattern of the phytoplankton community remains a challenging task when modeling lake ecosystems due to their extremely diverse behavior. This study represents a first dynamic modeling approach of functional groups of the phytoplankton community in deep, mesotrophic Lake Geneva using the water quality Model DYRESM-CAEDYM. Lake Geneva plays an important environmental role as it is the largest lake in central Europe in the peri-Alpine region, representing an essential resource for drinking water supply. This region is particular sensitive to climate change and it is hypothesised that more episodes of harmful cyanobacteria outbreaks will occur under warmer climatic conditions, which may lead to negatife impacts on its water quality and the public health. Our aim was to produce an accurate predictive management tool for Lake Geneva and to assess the ecological state of the lake under present as well as under future climatic conditions, with focus on the cyanobacteria community and its successional sequence. For this purpose, morpho-functional groups of phytoplankton specific for Lake Geneva were identified. The resulting groups were separated based on their functional ecological behavior but also on their morphology, as well as links to abiotic seasonal conditions. The simulations demonstrated the close relationship of successional sequences with mixing and stratification in Lake Geneva, which were strongly seasonally driven. Morpho-functional groups appear to be an appropriate level of state variable representation in this type of modeling approach to enable valuable insights into emerging environmental drivers such as climate change.

S15.2

Insights into the dynamics of a toxic dinoflagellate population and shellfish toxicity in a tropical estuary through a coupled physical-population-toxin model

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Knowledge on the mechanisms driving tropical Harmful Algal Blooms (HABs) are limited relative to the well-studied temperate HABs. The interaction of temperature, cysts (for certain species) and large-scale transport are some of the key processes in temperate HABs. In the Philippines, HABs occur not along long open coastlines, but in embayments that are highly influenced by run-off and stratification. These embayments are also the sites of cultured or wild harvest shellfish and other aquaculture activities. Sorsogon Bay in the Philippines has experienced prolonged shellfish bans due to blooms by *Pyrodinium bahamense* var. compressum (Pbc) severely affecting the fisheries industry in this area. A novel integrated model was developed that mechanistically captures the interactions between hydrodynamic conditions, nutrients, the life history (cells and cysts) of Pbc, as well as the cultured shellfish within the bay and their ensuing toxicities due to ingestion of toxic Pbc cells and cysts. The model is modularly composed of a 3D hydrodynamic model, a watershed nutrient and diffusion model, a Pbc population model and a shellfish toxin model. The linkages between the bloom and decline of the Pbc population with shellfish toxicity as affected by temperature, salinity and nutrients were explored with the model. Comparisons with field results highlighted the role of stratification and nutrients in Pbc dynamics, and the importance of cysts in both Pbc blooms and shellfish toxicity patterns. This model aims to serve as a decision support tool for managing HAB occurrences in tropical embayments typically affected by cyst-forming dinoflagellates in the Philippines.

S15.3

Estuarine circulation and the initiation of harmful algal events in Killary Harbour, Ireland: Dancing with the tides revisited

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Killary Harbour, a fjord on the west coast of Ireland, is a site of shellfish aquaculture that undergoes harvest closures in many summers due to contamination of rope-cultured mussels with DSP toxins deriving from *Dinophysis*. The harmful events are caused by the transport into the estuary of populations of *D. acuminata* or *D. acuta* from near coastal waters on the continental shelf. This paper presents current profiler data from extended deployments inside and outside the estuary. Deployments inside the estuary show that variability in net sub-surface water influx at the mouth, and hence entrainment, is clearly linked to the spring-neap cycle with a frequency of 14 days. This is a response to the increased levels of vertical mixing experienced at spring tides, with the circulation showing the characteristics of a partially mixed estuary. Influx of sub-surface water, and entrainment, is affected to a much lesser effect by wind, and less still by the variability in freshwater inflow to Killary Harbour. Currents were large enough and in the correct direction, to transport *Dinophysis* populations the distances required to advect populations across the relatively shallow region from the continental shelf into Killary Harbour. Examination of over a decade of HAB monitoring results from three sites within the estuary shows that the onset of *Dinophysis* events is linked to the spring-neap tidal cycle and the modulation of entrainment. This allows an element of prediction of DSP and other contamination events in Killary, albeit over relatively short (~10-15 day) time-scales.

S15.4

Fluidics around algal cells

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Have you ever wondered how algae stay so clean, even though most non-living surfaces gather "dirt" and biofilm? Well, most flowering-plant leaves also stay clean. Under air, water and "dirt" films are repelled. Repulsion forces the water into droplets that easily roll off because these leaves are covered in hydrophobic nm- to µm- sized grooves and pillars, producing "superhydrophobicity" (SH) at the surface. Similarly, most algal cells bear a glycocalyx of organic fibrils that give surface structure, and are often hydrophobic. Glycocalyxes serve many functions, but whether they produce SH is poorly known. HAB diatom and dinoflagellate cultures were recently shown to *reduce* viscosity in laminar capillary flow, possibly by SH drag reduction, a phenomenon now well know from laminar flow in SH capillaries. SH coatings are being developed to prevent fouling of ships and aquaculture structures without using toxins, so this technology could help understand how algae defeat fouling.
Glycocalyxes are composed of exopolymeric substances (EPS), and algae sometimes make the water *more* viscous using this tightly and more loosely bound EPS. EPS is also sometimes sticky. Copepods have to spend time scraping sticky algal cells off their feeding appendages. Can SH cuticles on copepods help defeat sticky algal attack? On cells generally, glycocalyxes also serve in immunological-type recognition, binding and sensing. This may allow algae the tools to discriminate in their engineering of ambient fluidics, as part of their environment.
A working group has been formed in this field, open to interested scientists.

S16.1

Structural elucidation of palytoxin congeners in *Palythoa* sp., *Ostreopsis siamensis*, *O. ovata* IK2, and *O. ovata* AZ using liquid chromatography-mass spectrometry

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The dinoflagellates *Ostreopsis* spp. produce highly toxic palytoxin congeners and thus make up an important target for monitoring. Their wide distribution and broad genetic variations are well-known. On the other hand, the information on toxin structures is scarce, mainly due to difficult purification and the large and complex nature of the molecules. Applying LC-TOFMS to small and crude samples, we clarified structural features of 13 congeners: two from *Palythoa*, three from *O. siamensis*, three from *O*. cf. *ovata* IK2 strain from Japan, and five from *O*. cf. *ovata* AZ strain from the Gulf of Naples. Distinctive changes occurred in the following functional groups: *N*-containing terminal unit connected to C1, methyls on C3 and C26, hydroxyls between C15 and C20, hydroxyls on C42 and/or C46, and C115-amine. The toxin profiles could characterized strains, in addition to genetic types, and provided useful information for applying the ELISA or spectrometric methods. The toxin production markedly varied among strains and the structural changes strongly affected the potencies.

S16.2

Ostreopsis cf. *ovata* from the Mediterranean area: Variability in toxin profiles and structural elucidation of unknowns through LC-HRMSn

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Ostreopsis spp. once confined to tropical and subtropical areas have recently spread to temperate regions such as the Mediterranean and the Southern-Atlantic coasts of Europe with relevant negative impact on human health through skin contact and inhalation of toxic aerosols. Following *Ostreopsis*-related outbreaks, we developed a liquid chromatography-high resolution mass spectrometry (LC-HRMS) method for the analysis of algal samples. Our studies characterized *O.* cf. *ovata* as producer of palytoxin congeners, including a putative palytoxin and ovatoxins. Toxin profiles of different algal strains appear sometimes different both qualitatively and quantitatively and the presence of further new ovatoxins is emerging. Herein we report on a systematic study of over 40 Mediterranean strains of *O.* cf. *ovata*. All of them showed to produce ovatoxins although at different levels. Four types of toxin profiles were identified. Most of the strains produced ovatoxin-a as major component of the toxin profile while a significant number of strains did not produce ovatoxin-b and -c. Unique toxin profiles were found in a strain from the Adriatic coast of Italy, in which ovatoxin-f was the dominant toxin, and in a strain from the French Mediterranean coast, which produced only ovatoxin-b and c. On a per cell basis, toxin contents were in the range 5-230 pg/cell. In light of the structural complexity of ovatoxins, a LC-HRMS^n based approach was employed to gain structural insights on known and unknown components of toxin profiles that could be available in too small amounts to be studied by NMR.

S16.3

Toxin production in *Ostreopsis ovata* involves a new type of nonribosomal peptide synthase

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Polyketide synthases (PKSs) and nonribosomal peptide synthases (NRPSs) are well-known multienzyme complexes of bacterial and fungal secondary metabolism. NRPSs typically have condensation and adenylation domains, the latter of which determines the substrate specificity. In the last decade, PKS and NRPS genes have been identified in protists, including dinoflagellate species. A strain of the dinoflagellate *Ostreopsis ovata* that was sampled in Okinawa, Japan, produces several ovatoxin variants. Ovatoxin belongs to the same structural family as the extremely poisonous compound palytoxin. Sequencing of the *O. ovata* transcriptome by means of RNA-Seq revealed a wide range of genes encoding PKSs and NRPSs. Most interestingly, *O. ovata* possesses a new type of NRPS, which is characterised by an unusual monofunctional architecture. These enzymes form a dinoflagellate-specififc clade in phylogenetic reconstructions. Expression on the RNA level was confirmed by RT-PCR. Heterologous expression of identified adenylation domains in *Escherichia coli* followed by biochemical determination of substrate specificities showed that the new type of NRPS found in *O. ovata* is involved in the biosynthesis of Ovatoxin.

S16.4

Insights on the toxic effect of *Ostreopsis* cf. *ovata*: the role of direct/indirect contact with microalgal cells

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Ostreopsis cf. *ovata* is a harmful benthic dinoflagellate, widespread along most of the Mediterranean coasts. It produces several toxins and variable amounts of mucus that may totally cover substrates, especially during the stationary phase of blooms, when cells are aggregated into a mucilaginous matrix. Studies on different aspects of the biology and ecology of *Ostreopsis* spp. are increasing, yet knowledge on toxicity mechanism is still scarce. In particular, the potential active role of the mucilaginous matrix has not yet been proved, although when mass mortalities have occurred, organisms were reported to be covered by the typical brownish mucilage. In order to better elucidate toxicity dependence of direct/indirect contact with *O.* cf. *ovata* cells, we carried out toxicity bioassays exposing *Artemia salina* nauplii and ephyrae of *Aurelia aurita*, an innovative model organism, to several treatments of *O.* cf. *ovata* culture. Simultaneously, molecular and chemical analyses were performed to validate our methods and characterize the toxin profile. Our results have reported higher effects when a direct contact between model organisms and intact microalgal cells occurs ($LC_{50\text{-}48h}$ < 4 cells/ml on *A. salina*, $EC_{50\text{-}48h}$ = 9 cells/ml on *A. aurita*), although also growth medium devoid of microalgal cells but containing *O.* cf. *ovata* mucilage and filaments, caused significant toxic effects. Additionally, the connection between mucilaginous matrix and thecal plates, pores and trychocysts was explored by way of atomic force microscopy (AFM) to investigate cell surface at a sub-nanometer resolution, providing a pioneering description of the inner side of *O.* cf. *ovata* thecal plates.

S16.5

Palytoxin and okadaic acid co-exposure: *in vivo* and *in vitro* effects

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The increasing distribution of microalgae producing palytoxin (PLTX) or okadaic acid (OA) poses concern on their co-occurrence in seafood and risks for human health due to possible synergistic toxic effects. Thus, *in vivo* and *in vitro* studies were carried out to evaluate the toxic effects due to PLTX and OA co-exposure. Groups of 8 female CD-1 mice were orally administered with combined single doses of PLTX (30, 90 or 270 μg/kg) and OA (370 μg/kg), or with each toxin alone, recording toxicity signs and lethality for 24 h and 14 days. Lethality occurred only after PLTX (90 and 270 μg/kg) exposure, alone or combined with OA, also during the withdrawal period, with piloerection, paralysis and dyspnea as main toxicity signs. At 24 h, slight/mild ulcers and inflammatory infiltrates in the forestomach were noted only after PLTX (90 and 270 μg/kg) and OA co-administration. Gastrointestinal swelling and a dose-dependent decrease in liver weight, accompanied by a decreased glycogen in hepatocytes, were observed in mice co-administered with OA and PLTX (270 μg/kg) or with PLTX alone; no alterations were recorded after 14 days. *In vitro* studies on the human hepatic IHH cell line revealed a possible additive cytotoxic effect between PLTX (10^{-11} M) and OA (10^{-7}-10^{-6} M) after 24 and 48 h co-exposure. These results indicate a potential interaction of the two toxins that should be considered in their risk assessment as seafood contaminants.

S17.1

Understanding the niche of marine and freshwater harmful algae through the lens of eco- transcriptomics

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The availability of fully annotated genomes and the speed of whole transcriptome shotgun sequencing (RNA-seq) presents the opportunity to understand the niche of harmful algae through the lens of eco-transcriptomics –studies that explore gene expression–environment interactions. We have conducted transcriptomic studies of representative marine (*Aureococcus anophagefferens, Aureoumbra lagunensis*) and freshwater (*Microcystis aeruginosa*) harmful algae grown under conditions commonly present during HABs: low levels of inorganic nitrogen, inorganic phosphorus, and light as well as elevated levels of organic matter. Concurrently, ecosystem studies were performed during which transcriptomes were sequenced from *in situ* field populations before, during, and after HABs, as well as from experimental manipulations of field populations. Comparisons of ecosystem-based transcriptomes to culture-based results have been used to identify specific gene pathways that are up-regulated to facilitate bloom formation. In some cases, commonalities exist among harmful algae: up-regulation of membrane bound nutrient transporters in response to low inorganic nitrogen or inorganic phosphorus levels. In other cases, species specific responses have been observed: up-regulation of enzymes involved in the degradation of organic matter by *A. anophagefferens* in response to resource poor environments. Finally, these eco- transcriptomic investigations have proven useful in assessing factors regulating toxin production in HABs. We have specifically determined that expression of genes associated with microcystin production in *Microcystis* are up- and down-regulated in response to nitrogen supply with high inorganic nitrogen levels facilitating up-regulation of microcystin synthease (*mcy*) genes and elevated microcystin content. The prospects of identifying an overarching HAB niche via eco-transcriptomics will be discussed.

S17.2

Molecular mechanisms of the interaction between the diatom *Phaeodactylum tricornutum* and the dinoflagellate *Alexandrium tamarense*

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The succession of dominant phytoplankton communities plays an important role during the harmful algae bloom (HAB). However, the molecular mechanism of the ecological succession remains unclear, such as the most commonly occurred succession of diatoms and dinoflagellates. Hence, we investigated the molecular responses of a diatom *Phaeodactylum tricornutum* during the co-cultivation with a dinoflagellate *Alexandrium tamarense*. The growth of *P. tricornutum* was significantly inhibited within 24 h of co-cultivation. The organelles such as the chloroplast and mitochondrion in *P. tricornutum* were severely damaged, including the blurring or disintegration of membrane and the deformation of organelles. Transcriptional changes of *P. tricornutum* after co-cultivation were analyzed by RNA-sequence. Transcription levels of genes involved in glycolysis, TCA cycle, β-oxidation and oxidation phosphorylation were downregulated, similar to the carbon fixation and photosynthesis, indicating that the capabilities of energy metabolism and uptake of carbon dioxide were inhibited. And several proteins associated with check points and cell cycle were also downregulated, suggesting the occurrence of the suppression of DNA replication and cell division. Moreover, upregulation of ABCB1 transporter implicated that certain allelochemicals could be released from *A. tamarense* and played a role in the interaction between diatoms and dinoflagellates. Furthermore, a predicted leucine-rich repeat receptor-like kinase flagellin-sensitive 2 (FLS2) was also dramatically upregulated, indicating another pathway involved in the interaction different from the allelopathy. Our results shed light on the molecular mechanisms of the interaction between diatoms and dinoflagellates, thus contributing to the explanation of the succession of phytoplankton communities during HAB.

S17.3

Transcriptome analysis of *Pseudo-nitzschia australis* yields insights on nitrogen assimilation and domoic acid biosynthesis in this toxigenic diatom

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Numerous physiological studies have pointed to a dependence of domoic acid biosynthesis on the growth status of *Pseudo-nitzschia* spp, such that DA accumulation is generally enhanced during growth limitation by a range of stressors. While DA accumulation can be affected through manipulation of growth rate in this diatom genus, such manipulations confound interpretation of DA biosynthetic patterns and pathway reconstruction. Here we report on transcriptomes generated from a Monterey Bay isolate of *P. australis* (10249_10AB) pre-acclimated to and grown with different N-source supplementation (NO3, NH4, UREa, GLUtamate) provided at a common supply rate (0.27/d of 40 µM-N and f/2 levels of other constituents). Total DA accumulation varied by a factor of two being highest in the GLU culture (0.25 pg/cell) with a rank order of GLU>NO3>NH4>URE. DNA-free RNA was submitted to the National Genome Resource Center for RNA-seq library construction, sequencing and annotation as part of the **M**arine **M**icrobial **E**ukaryote **T**ranscriptome **S**equencing **P**roject (Project IDs: MMETSP_139_2, 140_2, 141_2, 142_2). An average of 23,317 contigs (2178 bp median length) were machine assembled from each library with 80% having defined protein coding sequences. Of these putative genes an average of 3899 (22%) sequences per library had significant homologies ($E<10^{-10}$) to known proteins enabling pathway reconstruction. *P. australis* expressed transcripts encoding enzymes required for complete mevalonate (*hmgr*-dependent) and methyl-erithrytol phosphate (*dxs*-dependent) pathways for isoprene generation. Strong correlation of dxs and argD transcript levels with DA accumulation point to the MEP and ornithine cycle as respective sources of the isoprene and pyrrolidine moieties of DA.

S17.4

Comparative transcriptomic analysis of three toxin producing *Karenia* species

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Transcriptomic data for three *Karenia* species *(K. brevis, K. papilionacea, K. mikimotoi)* were compared to investigate putative genes involved in toxin biosynthesis. Recent results have shown that *K. papilionacea* also produces brevetoxin (PbTx-2 only) and increases production of this ladder-frame polyether during hypoosmotic stress, as is observed in *Karenia brevis*. In contrast, *K. mikimotoi* does not make brevetoxin and instead produces gymnocin, another type of ladder-frame polyether. Ladder-frame polyethers are a type of polyketide that are thought to be synthesized intracellularly using polyketide synthases. We found 101 putative polyketide synthase (PKS) unigenes in the *K. brevis* transcriptome. Approximately 130 million short reads for *K. papilionacea* and for *K. mikimotoi* were aligned to the reference *K. brevis* transcripts. We identified 13 PKS unigenes that were only expressed in the two brevetoxin-producing species. A BLASTn search querying the transcriptomes of *Karlodinium micrum*, *Alexandrium fundyense* and *Prorocentrum minimum* for these 13 PKS unigenes yielded no hits, thus suggesting these unigenes are unique to *K. brevis* and *K. papilionacea* and may represent potential steps in the brevetoxin synthesis pathway. We have also observed that environmental factors influence the type of ladder-frame polyether backbone structure produced. In *K. brevis*, variation in light exposure (i.e., longer day length) significantly increased the production of brevenal and PbTx-2. Increased light intensity, however, had no effect on brevenal production or PbTx-2.

S17.5

Dinoflagellate transcriptomics - hunting for potential marine biotoxin synthesis genes

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Dinoflagellates are unicellular eukaryotic primary producers. They occupy an important niche in marine and freshwater environments, and have ecological and economic impacts, for example, through the formation of harmful algal blooms. Many harmful algal bloom-forming species produce toxins with ecosystem-wide and human health impacts. Structural elucidation of many toxic compounds produced by dinoflagellates suggests that they may be based on a polyether ladder backbone, and that polyketide synthase enzymes may have a role in their biosynthesis. In this study, we assembled *de novo* transcriptomic gene catalogues of dinoflagellates of the orders Gymnodiniales and Gonyaulacales. Using bioinformatic tools such as BLAST2GO and HMMER, we identified genes potentially involved in polyketide biosynthesis. Bioinformatic analysis revealed a vast diversity of polyketide synthase genes encoding ketosynthase domains and ketoreductase domains. The ketosynthase domain encoding transcripts were found to form distinct clades in the phylogenetic analysis, in comparison to similar genes from other organisms, some of which was unique to toxin producing strains. The results presented here are a step forward towards recognising the genes encoding critical steps in toxin biosynthesis and designing tools to monitor for and mitigate human illnesses due to harmful algal blooms.

S18.1

Mitigating fish-killing algal blooms: clay revisited to remove ichthyotoxins

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Mitigation of ichthyotoxic algal blooms through clay flocculation has been widely explored, yet the potential of clay minerals to adsorb dissolved ichthyotoxins has received little attention. Previously (ICHA15), we demonstrated removal of *Prymnesium parvum* toxicity towards the gill cell line RTgill-W1 by Phoslock™ and have since worked on refining clay type and dosage. However, we failed to significantly reduce toxicity with Phoslock™ in high density *Prymnesium* cultures at pH above 10.We therefore explored 12 commercially available Australian (two zeolites, four kaolins and six bentonites) and two Korean yellow loess clays to remove *Prymnesium* toxicity at pH 7 and 9. At pH 7, bentonite clays displayed the highest removal of ichthyotoxicity (54% gill cell viability increase compared to control), followed by kaolins and zeolites (+26 and +22%, respectively), but Korean loess proved ineffective. At pH 9, kaolin, loess and zeolite exacerbated toxicity (-11, -15 and -17% viability loss compared to control). Toxin removal by Phoslock™ was also significantly less effective at elevated pH (9% viability increase vs. 57% at pH 7), explaining previous results with dense laboratory cultures. By contrast, calcium-bentonites performed better at higher pH (complete removal of ichthyotoxicity), outlining their potential as rapid response tools during high density fish-killing algal bloom events. We are currently successfully extending our application of clays to adsorb ichthyotoxins from other species such as *Karlodinium veneficum* and are investigating their use to remove reactive oxygen species (as O_2^-) generated by *Chattonella marina*.

S18.2

Manipulating phytoplankton flora to harmless diatoms through germination of resting stage cells by sediment-lift to euphotic layer in coastal sea

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There is an urgent need for bloom controlling strategies due to huge fishery damages by harmful algal blooms (HABs). It is empirically known that HABs by flagellates such as *Chattonella* and *Karenia* have occurred when diatoms are scarce in water columns. Diatoms produce resting stage cells under nutrient-depleted conditions, and rapidly sink to sea bottom and disappear from the water columns. Giving enough light to the abundant diatom resting stage cells at sea bottom is considered to enhance their germination and resultant vegetative cells are expected to rapidly proliferate in the surface water and to overwhelm harmful flagellate populations by the exhaustion of inorganic nutrients (N and P). We tried to lift surface bottom sediments into surface water with a pump and suspend diatom resting stage cells in euphotic layer in the coastal sea of Saiki Bay, the Seto Inland Sea, Japan, in September 2012 and May 2013. In the September 2012 trial, the diatoms of *Chaetoceros* spp. and *Skeletonema* spp. markedly increased from $<10^3$ cells mL^{-1} to 2 ~ 3 x 10^3 cells mL^{-1} within a few days in the water column. In the May 2013 trial, mainly *Chaetoceros* cells increased from about 50 cells mL^{-1} to 120 cells mL^{-1} in surface water despite under severe nutrient-depleted conditions (N <0.5μM, Si <5μM). In a neighboring "control" bay, no phytoplankton increase was detected with no sediment lifting. It is hopefully suggested that sediment-lift is a possible strategy for manipulating the phytoplankton flora to desirable diatoms to prevent harmful flagellate blooms.

S18.3

Growth, competition and grazing control of the potentially harmful dinoflagellate *Lingulodinium polyedrum* in a natural plankton community

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Population dynamics of potentially harmful dinoflagellates are regulated both 'bottom-up' by resources (e.g. concentrations of dissolved nutrients) and 'top-down' by zooplankton grazing. In the present study we investigated 'bottom-up' and 'top-down' effects on the population dynamics of the red tide dinoflagellate *Lingulodinium polyedrum* in coastal waters of Southern California, USA. Two laboratory experiments were conducted using a natural plankton community off the coast of Los Angeles. We manipulated the ratios and concentrations of dissolved nutrients by adding either phosphate, nitrogen or both to the natural seawater. In a second approach, the effect of the presence of potential consumers on growth and competitive behaviour of *L. polyedrum* was investigated by manipulation of the natural grazer assemblage and the addition of the heterotrophic dinoflagellate *Noctiluca scintillans*. The results indicate that *L. polyedrum* may successfully compete with dominant diatoms under low nutrient conditions. Furthermore, natural zooplankton grazers (63 - 110µm) decreased nanophytoplankton (< 20µm) and thus promoted the growth of *L. polyedrum* indirectly by competitive release. In turn, the heterotrophic dinoflagellate *N. scintillans* had a strong grazing impact on the *L. polyedrum* population, indicating that *L. polyedrum* growth can be controlled by zooplankton grazing. A subsequent laboratory experiment investigating grazing of *N. scintillans* on a gradient of different cell concentrations of *L. polyedrum*, however, indicated a grazing inhibition above a particular threshold concentration, which may increase the chance of a bloom.

S18.4

Examination of the fungal and bacterial populations on rotting barley straw used to control cyanobacterial blooms

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Control of cyanobacterial bloom formation using chemicals such as copper sulphate can have damaging environmental consequences. An alternative is the use of rotting barley straw, the efficacy of which has been previously confirmed. This study showed that prolonged incubation of sterile straw did not render it active at inhibiting the growth of the test cyanobacteria, conclusively demonstrating for the first time that straw activation requires microbial activity. This project was the first to examine microbial diversity on rotting barley straw and revealed a complex population of bacteria and fungi, many of which have the capacity for lignin degradation. This observation provides support for the hypothesis that lignin derivatives released during the breakdown of straw may be responsible for inhibiting cyanobacterial growth.

S18.5

Hydraulics and barley straw (*Hordeum vulgare*) as effective treatment options for a cyanotoxin-impacted lake

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Many freshwater and brackish systems are experiencing high nutrient loads and summer cyanobacteria blooms. In Maryland, USA, Lake Williston is surrounded by fertilized cropland, resulting in high nitrate (>600 uM) and phosphate (up to 10 uM) concentrations, as well as atmospheric ammonia (~80 uM) deposition. These elevated concentrations support dense, toxic blooms of *Microcystis aeruginosa* and other cyanobacteria, with microcystin (>1 ppm) (and anatoxin) routinely exceeding WHO standards for recreational use. Mitigation of lake cyanobacteria was attempted through hydraulic flushing and deployment of barley straw (*Hordeum vulgare*) in the 52 acre lake and feeder streams. Fall draining of the lake (80% volume reduction) exposed the lake's sediments and overwintering sedimented cyanobacteria to freezing conditions, ideally rupturing cells to limit subsequent summer blooms. This was followed by early spring deployment of barely straw bales (550/52 acres) throughout the system. Cores collected from the drained lake and exposed to slow increases in water temperatures, light, and nutrients showed no *M. aeruginosa* emergence/growth. Subsequent summer cyanobacteria growth was also limited, with 10-100 fold lower *M. aeruginosa* abundances and near absence of microcystin. The reductions could be partially explained by barely straw decomposition as field-collected samples in barley straw-rich areas inhibited growth of *M. aeruginosa* LE-3, likely attributed to polyphenolics released from the decomposing straw. Enhanced decomposition has been facilitated through the addition of white rot fungi, with similar strong inhibitory responses for *M. aeruginosa*, indicating future management might include fungi additions to deployed bales to foster even greater cyanobacteria loss.

S19.1

Novel toxins from the New Zealand red tide dinoflagellate *Karenia brevisulcata*

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A red tide dinoflagellate bloomed in Wellington Harbour, New Zealand in 1998. This caused mass mortalities of fish and invertebrates within the harbour. Over 500 cases of human respiratory distress were also reported, with symptoms including a dry cough, severe sore throat, and headache. The causative dinoflagellate was identified as a new species, *Karenia brevisulcata*, and a cell extract showed strong mouse lethality and cytotoxicity. The extract was partitioned between organic solvents and aqueous methanol under neutral and acidic conditions. From the neutral lipophilic fraction five novel toxins were identified, which we have named brevisulcenals (KBT). Isolation was guided by cytotoxicity against mouse leukemia P388 cells. The sodiated cations of KBTs in MALDI mass spectra were observed at over *m/z* 2000. The most abundant toxin, KBT-F was determined to be a large polycyclic ether compound with 24 ether rings, including dihydrofuran, 13 hydroxy groups, and a 2-methylbut-2-enal side chain that is similar to those of gymnocins produced by *Karenia mikimotoi*. From the hydrophilic acidic fraction another novel set of toxins were identified, and these were called brevisulcatic acids (BSXs). BSXs-4 and -5 have a γ-lactone as the 5-membered A-ring while BSXs -1 and -2 are the seco acid analogs. The BSXs consist of nine contiguous ether rings and contain a 9-membered ether ring within the central portion of the molecule. Its presence confounded the spectral interpretation of the BSX toxins. The structures of BSX-4 and -5 are similar to that of brevetoxin A. BSXs-4 and -5 also showed cytotoxicity against neuro2A cells analogous with brevetoxins and ciguatoxins.

S19.2

Fatty acid synthesis and amphidinol synthesis are metabolically linked in *Amphidinium carterae*

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We undertook Illumina RNA sequencing of two species known to produce PKS-derived toxins: *Amphidinium carterae*, and *Karlodinium veneficum*, and a third non-toxic species *Akashiwo sanguineum*. Both toxin and fat biosynthetic genes would likely contain KetoSynthase domains and previous results suggested only single KS domain genes would be present in dinoflagellates. Surprisingly multidomain PKS, NRPS, and hybrid PKS / NRPS genes ranging from 8-15 kb were found from all three species. Six different multidomain genes were found: one contained only PKS domains, three were hybrid NRPS /PKS genes, and two were NRPS genes. Introns were found in PCR products amplified from genomic DNA templates in *A. carterae* for all multidomain KS genes, although the NRPS genes were not tested. Similarly, all categories but the NRPS had at least one species with a partial characteristic dinoflagellate spliced leader sequence. Sequence assembly or coverage for these large transcripts required iterative assembly techniques in several cases. Phylogeny of KS, adenylation, AT, and KR domains from the complete dataset suggested the multidomain genes were acquired as such, rather than derived from single domain genes. However, the majority of sequences with PKS or NRPS domains were encoded as single domain genes and appeared to be quite diverse in these species. Using a known fatty acid synthesis inhibitor, cerulein, ^{14}C acetate *in vivo* incoporation into fatty acids and amphidinol were completely abolished in concert. The differences between toxin producing and nontoxic species in the multi KS gene could be traced to a missing AT domain.

S19.3

Evaluation of antibody and DNA-aptamer magnetic bead captures of saxitoxin with HPLC-MS/MS in human urine

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Saxitoxin is a potent neurotoxin that is present in harmful algal blooms and continues to be of public health interest due to regional poisonings following ingestion of contaminated shellfish. Current methods to detect saxitoxin in clinical samples have been used to confirm human saxitoxin exposure cases as well as identify cases, although similar in symptomology, not attributed to significant concentrations of saxitoxin. In recent years, more selective approaches to sample extraction have been applied to shellfish and seawater using antibodies and DNA-aptamers. The monoclonal antibody used here has been developed by an independent laboratory to have high selectivity for saxitoxin with IC_{50} values of 5.77 ng/mL. The DNA-aptamer investigated was commercially synthesized based on a novel sequence shown to bind saxitoxin with little cross-reactivity to other marine toxins. Previous work performed by our group has shown the feasibility of using magnetic beads for the capture and detection butyrylcholinesterase adducted nerve agents. Two new methods that use either an antibody or aptamer attached to a magnetic bead for capture and recovery of saxitoxin from urine are evaluated and compared to the current SPE LC-MS/MS analytical approach. The resulting highly specific and sensitive assay will be used to diagnose future exposures, complementing the many shellfish monitoring programs worldwide.

S19.4

Elucidating the origin of tetrodotoxin in New Zealand biota

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The ultimate origin of tetrodotoxin (TTX) remains one of the greatest mysteries in marine natural toxins research. The phylogenetic diversity of organisms containing TTX suggests an exogenous (most likely microbial) source of TTX, however, there is little conclusive evidence to support this.
In 2009, the grey side-gilled sea slug (*Pleurobranchaea maculata*) was found to contain extremely high concentrations of TTX in New Zealand. Since this initial detection, TTX has been identified in an additional 10 organisms in New Zealand, including very high concentrations in a flatworm, *Stylochoplana* sp., and lower levels in the bivalve *Paphies australis*, which is a maihangi kai (traditional food-source). A multi-faceted approach has been used to investigate the origin of TTX in these organisms. This involved; extensive environmental surveys, intensive bacterial culturing efforts, manipulative laboratory feeding studies, localization of TTX within organisms using immunohistological methods, the development of a new chemical method to explore precursor/ degradation products, and molecular approaches to investigate the diet of *P. maculata.*
Collectively these data strongly indicate a dietary source, most likely from a benthic micro-organism. To date we have been unable to definitively identify this organism/s, but our data suggests we may need to explore currently unknown pre-cursor molecules rather than only TTX.

S20.1

Transcriptional analysis of possible feedback mechanisms of lytic compound production in *Alexandrium fundyense*

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The Harmful Algal Bloom (HAB) species *Alexandrium fundyense* is responsible for outbreaks of paralytic shellfish poisoning in the marine realm of temperate waters. Unknown lytic compounds produced by *A. fundyense* are proposed to facilitate the formation of these toxic blooms by reducing losses due to competition and grazing. Nevertheless, as a survival mechanism, such chemical defence against consumption must be metabolically efficient enough to confer a selective advantage ensuring success among other potential prey organisms. We therefore identified possible feedback mechanisms whereby *A. fundeyense* might gain an advantage from lytic compound production on the molecular level. Based on a comprehensive cDNA-library, we compared the transcriptional response towards a protistan grazer (*Polykrikos kofoidii*) of an *A. fundyense* strain capable of lytic compound production versus that of a strain lacking this ability. We further highlight genes involved in secondary metabolite synthesis that are differentially expressed between these two strains. Our results strongly suggest that the action of lytic compounds produced by *A. fundyense* is associated with induction of a wide range of cellular functions. Significantly, lytic compound production seems to be a trait with more far- reaching consequences than as only a release from top-down grazing pressure. In summary, our results provide novel insights and issues for consideration regarding the autecology of this prominent HAB-species.

S20.2

Regulation of gene expression by light and nitrogen source in *Chattonella subsalsa* and *Heterosigma akashiwo*

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The raphidophytes, *Heterosigma akashiwo* and *Chattonella subsalsa* are globally distributed harmful algal species, causing massive fish kills in coastal environments. These species often co-occur, and recent research suggests that nitrogen availability or the relative abundance of nitrogen sources may play a role in species selection during the initial stages of a bloom. However, little is known about the molecular mechanisms that underlie their response to environmental conditions. Here, we sequenced the transcriptomes from *C. subsalsa* (CCMP2191) and *H. akashiwo* (CCMP2393) cultured under four conditions: High and low light intensities, and with nitrate or ammonium as a nitrogen source. Transcriptome libraries were constructed and sequenced using Illumina sequencing through the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) for *C. subsalsa* and at the Delaware Biotechnical Institute DNA Sequencing and Genotyping Facility for *H. akashiwo*. Sequences were assembled and differentially expressed transcripts for each treatment were identified using RNA-Seq by Expectation Maximization (RSEM) software. Differentially expressed genes were then verified by quantitative real-time PCR. This sequencing project will provide fundamental data from which to investigate transcriptional regulation of growth and toxicity in raphidophyte species, and ultimately enhance the development of ecological models of harmful algal bloom development.

S20.3

Transcriptomics of the dinoflagellates and identification of differentially expressed genes respond to algicide copper sulfate

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Dinoflagellate genomics is an important subject to gain molecular insights of red tide outbreaks. In this study, we applied RNA-seq technology to reveal the transcriptome data of four dinoflagellates, including *Amphidinium carterae*, *Cochlodinium polykrikoides*, *Prorocentrum micans* and *P. minimum*. All assembled contigs of each species were annotated with NCBI NR database, GO database, and KEGG database, respectively. The GO terms as well as the KEGG pathway of all the transcriptome were generalized for each species. Moreover, we further analyzed the transcriptome response of *P. minimum* and *C. polykrikoides* exposed to algicide copper sulfate ($CuSO_4$). Microarray analysis revealed that approximately 8.6% genes of tested probe of *P. minimum* responded to $CuSO_4$. As for *C. polykrikoides*, RNA-seq result revealed the differential expressed genes; around 21.2% of transciptome responded to $CuSO_4$ exposure. The regulated genes were further analyzed by GO terms, KEGG pathway, and manually analysis of some specific genes. Some of the genes that related to photosynthesis, mitochondria, copper binding or transporting were identified. Additional experiments showed that the photosynthesis pathway was considerably inhibited, and reactive oxygen species (ROS) production was significantly increased by $CuSO_4$ exposure in *P. minimum* as well as in *C. polykrikoides*. These results suggest that the dinoflagellate photosystems may be severely damaged when exposed to the algaecide, contributing to the dinoflagelalte bloom killings.

S20.4

Transcriptome analysis of *Heterocapsa circularisquama* in response to algicide TD49

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Harmful algal blooms (HABs) became a worldwide phenomenon which causes red and green tide in waters. Mechanical, chemical and biological controlling methods have been used to manage the algal blooms. TD49, which has a basic structure of thiazolidinedione (TD) compound, showed an effective and species-specific algicidal effect on harmful algal species. Demonstrating a selective algicidal effect of TD on the HAB, transcriptome analysis of *H. circularisquama* was investigated. RNA-sequencing was conducted in two conditions *H. circularisquama* treated with TD49 and control. The contigs were assembled and total of 155,399 transcripts were collected. Among all of its transcripts, 10,505 transcripts showed change more than two fold. To examine the mechanism of TD49 on *H. circularisquama,* well-known target genes of transcription factor PPARγ and other metabolic pathways were examined. Numerous genes that indicate lipid metabolisms were found, but few genes showed differences in their expression levels. The up-regulation of phospholipase which hydrolases lipid may weaken the cell membrane was revealed. In addition, up-regulation of genes related with antioxidant, cell death and heat shock proteins could explained that TD49 may induce the cell stress causing cell damage leading to a cell death. This transcriptome data of several genes were confirmed by qRT-PCR.

S22.1

Pinnatoxins A and G and the 28-*O*-palmitoyl ester of pinnatoxin G exhibit different activity on embryonic and mature nicotinic acetylcholine receptors

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Pinnatoxins belong to an emerging class of potent fast-acting marine phycotoxins of the cyclic imine group. Studies on their biological effects have been favored by the development of robust synthetic approaches. With synthetic pinnatoxins A and G, a detailed study has been performed *in vitro* and *in vivo* using electrophysiological, biochemical and molecular modeling methods providing conclusive evidence for its mode of action as potent inhibitors of embryonic and mature nicotinic acetylcholine receptors (nAChRs) of the neuromuscular junction. Interestingly, synthetic 28-*O*-palmitoyl ester of pinnatoxin G was about 325 times less active than pinnatoxin G on *Torpedo* nAChRs. The bases of such difference, evidenced by molecular modeling, are due to the steric hindrance between the 28-*O*-palmitoyl ester group and the residue in position 57 (Asp for δ subunit and Glu for γ subunit), which forces the ligand to adopt a less favorable conformation in the binding site. Pinnatoxins also affected spontaneous movements in chick embryos *in ovo*, and induced a marked decrease in skeletal bone development.

S22.2

Mechanism of action of pinnatoxins E, F and G

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Pinnatoxins E, F and G are the most recently discovered isomers of the cyclic imine pinnatoxin family, and are produced by the dinoflagellate species *Vulcanodinium rugosum*. These toxins were originally found in New Zealand, but have since been shown to occur around the world. Other toxins that belong to the cyclic imine group are highly toxic in *in vivo* rodent assays and cause toxicity by antagonising nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs) in the central nervous system and at the neuromuscular junction. However, the mechanism of action of the pinnatoxins is less well known. The aim of the present study was to investigate the mechanism of action of pinnatoxins E, F and G. In skeletal muscle preparations, pinnatoxins E, F and G were found to be potent blockers of neuromuscular transmission *in vitro*, with IC_{50} values in the low nanomolar range. Intracellular recordings in these preparations revealed that pinnatoxins F and G exhibit characteristics of post-synaptic nAChR antagonists. nAChR binding was confirmed and directly visualised using a fluorophore conjugated pinnatoxin F derivative and unlabelled nAChR antagonists in muscle sections containing fluorescent motor nerves. Radioligand binding assays revealed that pinnatoxins E, F and G not only bind to muscle type nAChRs, but also heteromeric and homomeric neuronal nAChR subtypes. The current studies show that pinnatoxins E, F and G are potent nAChR antagonists, both at muscle type and neuronal receptors.

S22.3

Brevetoxin metabolism and physiology – a freshwater model of morbidity in endangered sea turtles

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The dinoflagellate *Karenia brevis* is a key organism present during harmful algal blooms (HABs, red tides) which are increasing in frequency and duration worldwide. *K. brevis* produces a suite of neurotoxins which are collectively referred to as brevetoxins (PbTx). Brevetoxin exposure affects marine life by interrupting neurological function, decreasing immune function, and inducing inflammation. Brevetoxicosis is difficult to treat in endangered sea turtles, as the physiological impacts have not been fully investigated and the magnitude and duration of PbTx exposure is generally unknown. Freshwater turtles (*Trachemys scripta)* are being used as a model system for experimental toxin exposures. Analyzing uptake, tissue distribution, routes of excretion, immune function and neurological responses will give us insight into the fate of PbTx in vivo. Turtles exposed to PbTx orally or intratracheally show PbTx distributes to all organ systems, however it concentrates in the liver, bile and feces, with less excreted in urine and plasma. Phagocytic activity decreases 24h post-exposure suggesting impact to the immune system. Preliminary results indicate turtle neurons are surprisingly resistant to PbTx, although the mode of action is the same as in mammalian cells. Cell viability decreases in a dose dependent manner across PbTx concentrations from 100-1000nM; the LC50 was significantly higher than is seen in mammalian neurons. Understanding distribution, rates of clearance and effects of PbTx in these model turtles will allow us to design treatment strategies for threatened animals that become exposed during red tide events.

S23.1

Are the red and green *Noctiluca scintillans* both present in Manila Bay Philippines?

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Manila Bay located at the western side of the Philippines (14'15'–14'50'N and 120'30'– 121'00'E) facing the West Philippine Sea/eastern part of South China Sea, has a long history of toxic algal blooms caused by *Pyrodinium bahamense* since 1988. The green mixotrophic *Noctiluca scintillans* containing symbiotic alga *Pedinomonas,* has been observed to succeed this toxic *Pyrodinium* blooms until early 2000. Thereafter, the green *Noctiluca scintillans* seemed to have taken over the dominance of phytoplankton community mostly during the Southwest Monsoon period when *Pyrodinium* used to dominate. Sightings of red *Noctiluca*, which does not have the symbiont, were observed in March 2013 by two research institutions *i.e* University of the Philippines (UPMSI) and National Fisheries Research and Development Institute (NFRDI). There have been earlier reports of the co-occurrence of the green and red *Noctiluca* in other areas (*i.e* Arabian Sea, Vietnam, Indonesia), however, these sightings are the first in Manila Bay. Analysis of collected samples, some oceanographic data and satellite images that could explain the co-presence of green and red *Noctiluca scintillans* in a tropical system like Manila Bay will be utilized and explored.

S23.2

Rise and fall of toxigenic *Pseudo-nitzschia* populations and domoic acid observed by Environmental Sample Processor networks along the California coast

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We deployed the Environmental Sample Processors (ESPs) in Monterey and San Pedro Bays, regions of the California coast known for recurrent blooms of toxigenic *Pseudo-nitzschia* spp. Time-series assessments of *Pseudo-nitzschia* spp. abundance and domoic acid (DA) concentrations were generated from a series of month-long field campaigns over four years. Not surprisingly, significant variations in *Pseudo-nitzschia* spp. and DA were seen. High temporal sampling rates enabled by the ESP allowed observations of these changes over a range of environmental fluctuations. The local environmental conditions measured by the ESPs were placed within the context of larger-scale ocean observing systems that included remote sensing and *in situ* observations from ships, as well as moorings and autonomous underwater vehicles (AUVs). This contextual framework revealed key aspects of the environmental forcing, patchiness, and scale of the HAB events in the two coastal regions. This presentation will highlight results of this series of field studies, outline the challenges that remain, and introduce a new version of the ESP that can be fitted onto a small AUV to enhance HAB characterization over larger time/space scales than the current moored devices currently allow.

S23.3

Molecular phylogeny of benthic unarmored dinoflagellate *Amphidinium* spp. from Southeast Asia and adjacent areas

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Morphology and phylogenetic positions of benthic unarmored dinoflagellate *Amphidinium* spp. were examined by using light microscopy and molecular phylogeny to determine species distribution in Southeast Asia and adjacent areas. Surface sediment samples containing *Amphidinium* cells were collected from coasts of Australia, Indonesia, Japan, Malaysia, Palau, Vietnam, and 22 clonal cultures were established. Of these 15 cultures were identified to 5 previously described *Amphidinium* spp. based on morphology and phylogeny, i.e., *A. cupulatisquama* (2 strains from Japan), *A. gibbosum* (2 strains from Palau), *A. mootonorum* (1 strain from Japan), *A. thermaeum* (9 strains from Indonesia, Japan and Malaysia) and *A. trulla* (1 strain from Australia). Other 7 cultures could not be identified based on their morphology and phylogenetic positions, and tentatively designated as *Amphidinium* spp. 1–4. *Amphidinium* sp. 1 (2 strains from Palau) was smaller species ranging 12.0–22.5 µm long, similar to *A. thermaeum* but not related to the species in phylogenetic tree. *Amphidinium* sp. 2 (2 strains from Vietnam) measured 17.0–35.5 µm long, and was having multiple pyrenoids about 10. Cells of *Amphidinium* sp. 3 were 17.5–28.0 µm, and never formed immotile cells. *Amphidinium* sp. 4 (2 strains from Japan) ranged 17.7–27.5 µm. *Amphidinium* spp. have so far been identified as *A. carteriae* and *A. operculatum* based on cell sizes, many of our cultures from Southeast Asia and Japan were not related to the two species but to other species in the *Amphidinium* clade.

S24.1

Improved methodology for semi-automated identification of plankton and biovolume estimation using a Digital Imaging Flow Cytometer (FlowCAM®)

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In 1999 Dr. Christian K. Sieracki left the Bigelow Laboratory for Ocean Sciences and founded Fluid Imaging Technologies (FIT) with the intention to manufacture a digital imaging flow cytometer that he and his colleagues from Bigelow Laboratory had developed. Since then over 275 FlowCAMs in 40-plus countries have been put to use for the application of characterizing microorganisms in aquatic systems. Building upon advances in technology, input from the user community, and expanded resources, engineers at FIT have recently transformed the FlowCAM of 1999, designing the 4th Generation FlowCAM. Informally referred to as the 'High Sensitivity FlowCAM' (HSFC), the instrument provides more precise information over earlier versions – especially with regard to fluorescence data and size and concentration information. The HSFC has an improved method for sample introduction, and is simpler to use. Included among the next-gen FlowCAMs is an optics configuration (specific laser and optical filters) allowing for the detection of phycocyanin, thus providing for an improved method to identify and characterize cyanobacteria. In addition to a hardware overhaul, the image recognition algorithm and user interface for identifying and classifying organisms has been redesigned, as have the method to estimate biovolume. Included will be an overview of the various new designs, how they work, along with a review of data from the HSFC FlowCAM.

S24.2

Monitoring and managing benthic cyanobacteria blooms in the Hutt River, New Zealand: Issues, initiatives and lessons learnt

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The Hutt River is one of the most popular rivers for recreational use in New Zealand. However, in recent years risk to recreational users from blooms of the toxic mat-forming cyanobacterium *Phormidium* has increased. Since 2005, 11 dog deaths have been reported following contact with cyanobacteria in the Hutt River and, during the summer months, signs along the river bank warning people not to walk dogs near or swim in the river have become common. Over this time Greater Wellington Regional Council, in partnership with research providers, local councils and Regional Public Health, has continued to improve its monitoring of benthic cyanobacteria blooms and increased research into the contributing factors. Monitoring has evolved from a single transect of 10 observations of periphyton to semi-quantitative observations of cyanobacteria cover across four transects in riffle and run habitat. Another recent initiative is semi-quantitative assessments of fine sediment cover across the riverbed in response to growing scientific evidence that fine sediment may favour *Phormidium* establishment and growth. A number of management-related initiatives have also been developed and refined since 2005 including a regional 'toxic algae' response protocol setting out agency roles and responsibilities during bloom events, a river flow-based early warning system to signal the potential onset of blooms, and educational resources and seminars for river users. Content and management of health waning signage has evolved over the last nine years, with a key lesson learnt being that signage is only one part of a wider communications strategy needed to inform rivers users of potential risks from contact with *Phormidium.*

S24.3

Public health monitoring of cyanobacteria in Alberta, Canada

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Algal blooms in Alberta's freshwaters are predominantly due to cyanobacteria, some of which produce highly potent hepatotoxins called microcystins (MC) that are hazardous for health. Starting in 2010, 2397 water samples were collected from recreational beaches for the purpose of public health risk management. Laboratory analyses included: enumeration of cyanobacteria cell density; quantification of MC using protein phosphatase inhibition (PPI); enzyme-linked immunosorbent assay (ELISA); liquid chromatography/tandem mass spectrometry (LC-MS/MS); and quantification of MC synthase gene E (*mcy*E) with quantitative Polymerase Chain Reaction (QPCR). Reproducibility, accuracy, limit of detection, matrix effects, sensitivity, and specificity of the methods were determined. Linear regression was used to compare the methods. Estimated cost and time of laboratory methods was also compared. Public health advisories were issued for lakes based on visual inspection and in all cases cyanobacteria cell density exceeded a recreational water quality guideline. Cell counting was useful for determining the extent and community composition. MC levels exceeded a guideline in some of these lakes, but in most cases was not consistent with cell density. PPI and ELISA methods were considered for screening purpose and LC-MS/MS for confirmation. The QPCR method and the level of *mcy*E have the potential to be a predictive indicator of the expected cyanobacterial blooms in lakes.

S25.1

Long-term effects of human exposure to algal toxins - a review

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Adverse effects of human exposure to algal toxins have been known for centuries, but the problems with harmful algae seem to be increasing, and new toxic organisms and toxins are still being found. Authorities in many countries have programmes to protect against algal poisonings by applying guideline levels from the World Health Oganization. The acute effects of human poisonings with marine algal toxins are relatively well-known, and humans are, except for brevetoxins, exposed to these toxins mainly via food. The exposure routes for freshwater toxins are more diverse, including drinking water, bathing, and inhaling aerosols. The knowledge on the long-term effects on humans of chronic exposure to algal toxins is limited or absent with the exception of ciguatera toxins. No compilation of data or review on long-term effects exists. However, exposures to some algal toxins have been associated with chronic effects in humans or animals. For example, chronic exposure to microcystins has been linked to liver cancer and chronic liver injury. Exposure to the neurotoxic β-N-methylamino-L-alanine BMAA has been associated with subsequent neurodegenerative diseases and ciguatera toxins with long-term neurological problems in some people. Domoic acid (DA) exposure causes a chronic DA syndrome in sea lions. Our knowledge of the chronic effects of toxins like brevetoxins and saxitoxins is limited. No compilation of data exists, and the needed long-term human and animal epidemiology studies have not been done. We have compiled available information describing what we currently understand about the chronic effects from exposure to algal toxins in animals and humans.

S25.2

Biomarker for learning deficits caused by low level domoic acid exposure

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Domoic acid (DA) is a neurotoxin naturally produced by diatoms and commonly found in seafood. Routine monitoring programs have been affective at protecting seafood consumers from exposure to the high levels of DA that are known to cause overt signs of excitotoxicity such as disorientation, confusion and seizures. However, there are growing concerns about the effects of chronic low-level exposure for lifetime consumers of seafood such as razor clams that are known to contain low levels of DA throughout the year (regulatory limit = 20 μg DA/ g tissue). In the present study, we developed a mouse model of chronic low-level DA exposure to identify subclinical toxicity in the absence of overt signs of neuroexcitotoxicity. Mice were exposed to DA (≅ 0.7 μg/g per 1/wk for 25 wks IP) and then evaluated using open field activity and radial maze learning and memory test paradigms immediately following the 25-week exposure period and again after a 9-week recovery period (no DA). Hyperactivity and severe learning deficits were observed after 25 weeks of exposure. After the subsequent 9 weeks of recovery, these learning deficits were reversed, although performance latency (length of time required to make correct choices) was still impaired compared to control mice. Additionally, a DA-specific antibody was detected in serum of exposed mice and was temporally correlated with the learning deficits at 25 weeks; the antibody was not detected in serum after the 9-week recovery period, suggesting presence of the DA-specific serum antibody may serve as a biomarker of DA-induced subclinical neurotoxicity.

S25.3

Parallel monitoring of *Ostreopsis* cf. *ovata* blooms and human respiratory irritations in the NW Mediterranean

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Since the last decade, there has been an increase in the bloom occurrences of the toxic dinoflagellate genus *Ostreopsis* in temperate areas. In the Mediterranean Sea, massive blooms of *Ostreopsis* cf. *ovata* in some localities have been related to respiratory and dermatological symptoms in humans. Until now, the available health data comprise outbreaks of human affectation registered concurrently to high *Ostreopsis* cell densities. Because the blooms last for some months and symptoms are detected only occasionally, the direct link between the two kinds of events has not been clearly established yet. In order to address this discrepancy, in summer-autumn 2013, a joint epidemiology and ecology study was conducted in Sant Andreu de Llavaneres, a hot spot of *Ostreopsis* blooms in the NW Mediterranean Sea. Health disorders of a human cohort situated in front of the sampling station were continuously recorded along the whole *Ostreopsis* bloom period. In the water column, the highly variable cell numbers showed no link with human symptoms. In contrast, *O.* cf. *ovata* abundances in the macroalgae presented a well-defined temporal bloom pattern, and human health disorders peaked coinciding with the transition from the exponential to the stationary growth phase. We hypothesize that palytoxins, other secondary metabolites and/or potentially allergenic substances from the associated microbial community, could be released to the environment coinciding with that particular physiological state of the *Ostreopsis* cells. Thereafter, despite high cell densities were found in the macroalgae, the risk of potential respiratory impacts would be minor.

S25.4

Study of the combined toxicity of lipophilic phycotoxins on a human intestinal embryonic cell line

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Lipophilic phycotoxins can be found alone or in mixtures in bivalves filtering toxic phytoplankton. However, few toxicological data is available on the possible interactions between these phycotoxins and their risks for human health. In order to study their combined toxicity, we selected four main toxins: okadaic acid (OA), azaspiracid 1(AZA-1), pectenotoxin 2 (PTX-2) and yessotoxin (YTX). To avoid the use of transformed human intestinal cell lines, we chose to work with Human Intestinal Epithelium Crypt-like cells (HIEC), which offer characteristics similar to human intestinal crypts cells. Using both conventional cytotoxicity tests and High Content Analysis based approaches, various toxic effects (apoptosis, genotoxicity and cell cycle) were quantified following exposure to individual toxins and mixtures of toxins. Compared to the data that we previously obtained with the human intestinal cell line Caco-2 exposed to the same toxins (Ferron et al, 2014, *under review*), our results demonstrate differences in response between the two cell lines. Overall, HIEC were more sensitive. The combined effects of OA, AZA-1, PTX-2 and YTX were analyzed using a combination index method described by Chou and Tallalay (1984). According to the dose response curves, each binary toxin mixture was characterized by its effect (*i.e*. additive, synergistic or antagonistic). Based on an innovative approach, our results provide promising data for predicting the effects on human intestine following exposure to lipophilic toxins and mixtures of these toxins.

S25.5

An outbreak of paralytic shellfish poisoning in the Bay of Plenty, New Zealand

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During the week of 10 December 2012, at least 30 people became ill from consuming shellfish (mainly Tuatua *Paphies donacina)* collected recreationally from Bay of Plenty, New Zealand, beaches between Mt Maunganui and Papamoa. Fourteen people were admitted to hospital and three serious cases required treatment in the intensive care unit for several days but fortunately no deaths. High numbers of *Alexandrium minutum* were observed in the phytoplankton, the patients exhibited classic symptoms of paralytic shellfish poisoning (PSP), specimens from a batch that caused human poisoning contained high levels of STX and neoSTX and toxin residues were identified in the urine from an affected individual. This was the most serious PSP event that has been documented in New Zealand to date. Although a public health warning was in place, this was clearly ineffective in preventing people from harvesting dangerously contaminated shellfish. A contributing factor may be that the public had "health warning fatigue" because most PSP contamination events in the Bay are due to *Alexandrium catenella* blooms which predominately contaminate shellfish with lower toxicity analogues to just above the regulatory level.

S26.1

Spatiotemporal changes in the genetic diversity of harmful algal blooms caused by the toxic dinoflagellate *Alexandrium minutum*

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Organisms with sexual and asexual reproductive systems benefit from both types of reproduction. Sexual recombination generates new combinations of alleles whereas clonality favors the spread of the fittest genotype through the entire population. Therefore, the rate of sexual versus clonal reproduction has a major influence on the demography and genetic structure of natural populations. We addressed the effect of reproductive system on populations of the dinoflagellate *A. minutum*. More specifically, we monitored the spatiotemporal genetic diversity during and between bloom events in two estuaries separated by 150 km for two consecutive years. An analysis of population genetic patterns using microsatellite markers revealed surprisingly high genotypic and genetic diversity. Moreover, there was significant spatial and temporal genetic differentiation during and between bloom events. Our results demonstrate that (1) interannual genetic differentiation can be very high, (2) estuaries are partially isolated during bloom events, and (3) genetic diversity can change rapidly during a bloom event. This rapid genetic change may reflect selective effects that are nevertheless not strong enough to reduce allelic diversity. Thus, sexual reproduction and/or migration may regularly erase any genetic structure produced within estuaries during a bloom event.

S26.2

Quantitative detection of *G. catenatum* by qPCR based on the rDNA-ITS and *sxtA4* genes

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Blooms of *Gymnodinium catenatum* occur annually in southern Tasmania, particularly the Huon Estuary, D'Entrecasteaux Channel and Port Esperance. To detect the presence of *G. catenatum* in the environment a *G. catenatum*-specific quantitative PCR assays were established targeting the rDNA-ITS (ITS1-5.8S-ITS2) region and combined with a qPCR targeting the A4 domain of the saxitoxin synthetase gene (sxtA4). The *G. catenatum* rDNA-ITS primer specificity was tested against related gymnodinoids including *G. nolleri* and *G. microreticulatum* and were capable of detecting the presence of the saxitoxin-producing species in the water environment at concentrations below routine light microscopic detection. This assays are being developed as an early warning of the potential PST toxicity in the water environmental.

S26.3

Diversity of harmful unarmoured dinoflagellates from the Catalan coast, NW Mediterranean Sea

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Unarmoured species represent ~25% of the described dinoflagellates. However, their identification is challenging because the lack of a theca results in their deformation when fixed, and several of their key characters are difficult to observe in live specimens under the light microscope. Consequently, in analyses of fixed samples, unarmoured dinoflagellates, which include several harmful and/or toxic species, often remain unidentified or undetected. Although the composition of the phytoplankton along the Catalan coast, NW Mediterranean Sea, has been well studied since the 1960s, the applied methodologies usually impeded or hindered the identification of unarmoured dinoflagellates. In the present work, the diversity of unarmoured dinoflagellates in this geographic area was studied during 2011–2013 combining morphological observations of live specimens and genetic methods (single-cell PCR). This approach resulted in the detection of 60 unarmoured species, nine of which were potentially toxic species and six were high-biomass bloom producers. *Karenia umbella* and *Cochlodinium fulvescens* were reported for the first time in the Mediterranean, and *Cochlodinium polykrikoides*, *Karenia mikimotoi*, and *K.* cf. *papilionacea* along the Catalan coast. The blooms detected for *Barrufeta bravensis*, *Gymnodinium impudicum*, *Gymnodinium litoralis*, and *Karlodinium armiger*, whose type locality is in the NW Mediterranean Sea, highlight the importance of these unarmoured dinoflagellates and the need for further efforts to understand their role in the study area. Moreover, although the efforts conducted during the last decades, our findings point out the lack of knowledge about the worldwide distribution of many toxic and harmful species.

S26.4

Fragilidium fissile Balech (Dinophyceae): Life-history, morphology, phylogenetic position and feeding activity

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The mixotrophic dinoflagellate *Fragilidium* Balech has been reported rarely in taxonomic checklists, probably as a consequence of its confusion with the genus *Alexandrium*, and also the biological features are poorly known. In this study, we investigated the life history of mixotrophic dinoflagellate *Fragilidium* observed from Masan Bay, Korea and clarified the morphology and phylogenetic position using partial large subunit ribosomal DNA gene sequences. *Fragilidium*-like resting cysts characterized by transparent and round shape were isolated from sediment trap samples of Masan Bay, Korea. The isolated resting cysts germinated into gymnodinoid planozygote and directly formed the pellicle cyst, and then the thecate planozygote was observed after the germination of pellicle cyst. The gymnodinoid and thecate planozygotes were larger than vegetative cells. The vegetative cell was identified as *Fragilidium fissile* on the basis of theca morphology and tabulation; the plate formula is Po, Pc, 4', 8'', 10c, 6s?, 7''',2'''', 1p. Comparisons of partial large subunit ribosomal DNA gene sequences revealed that *F. fissile* is nested within *Fragilidium* and formed a strongly supported clade with *Fragilidium* cf. *duplocampanaeforme* and *Fragilidium subglobosum*. In addition, we first observed the feeding of *F. fissile* on *Alexandrium tamarense* and *Alexandrium catenella* and the clear visual evidence is provided in this study.

S26.5

Morphology and molecular characterization of the new planktonic phototrophic dinoflagellate *Gymnodinium smaydae n.* sp., from the coastal waters of Korea

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The marine phototrophic dinoflagellate *Gymnodinium smaydae* n. sp. is described from cells prepared for light, scanning, and transmission electron microscopy. Also, sequences of the small (SSU) and large subunits (LSU) and the internal transcribed spacer region (ITS1–5.8S–ITS2) of ribosomal DNA were analyzed. This newly isolated dinoflagelate possessed nuclear chambers, nuclear fibrous connective, an apical groove running in a counterclockwise direction around the apex, and a major accessory pigment peridinin. This dinoflagellate had a sharp and elongated ventral ridge reaching half way down the hypocone, unlike other *Gymnodinium* species. Moreover, displacement of the cingulum was 0.4–0.6 9 cell length while in other known *Gymnodinium* species it is less than 0.3 9 cell length. The sequence of the SSU, ITS1–5.8S– ITS2, and LSU rDNA region differed by 1.5–3.8%, 6.0–17.4%, and 9.1–17.5%, respectively, from those of the most closely related species. The phylogenetic trees demonstrated that the new species belonged to the *Gymnodinium* clade. Based on morphological and molecular data, we suggest that the taxon represents a new species, *Gymnodinium smaydae* n. sp.

S27.1

The exceptional 2013 bloom of *Dinophysis* in Scottish waters and its associated diarrhetic shellfish poisoning event

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During July 2013 approximately 70 people in south east England reported symptoms consistent with diarrhetic shellfish poisoning. The vast majority of cases occurred between 13 and 15 July. The cases were linked to the consumption of mussels originating from a particular harvesting area in the Shetland Isles Scotland. After these mussels were consumed, an unusually high toxin level was detected by the weekly regulatory monitoring programme. The negative publicity for the shellfish industry from this event was significant, with news reports on the BBC and other outlets reducing consumer confidence in Scottish shellfish. The biotoxin event was linked to an exceptionally rapid increase in the abundance of *Dinophysis* sp. in west coast Shetland waters, with cell abundances reaching ~ 8000 cells L^{-1} at some sites. The speed of increase in cell numbers was such that shellfish toxicity increased from sub threshold to high levels (~1500 µg/kg) in a time scale less than the one week resolution of regulatory sampling. As a result contaminated shellfish were marketed and consumed. The role of environmental factors in stimulating this bloom was investigated with analysis of wind patterns indicating that unusual changes in the direction of the prevailing wind was related to the statistically significantly elevated *Dinophysis* densities in 2013. It was also found that similar wind patterns generated high *Dinophysis* abundance in Shetland in 2006. A HAB early warning system for the Shetland isles and the incorporation of wind forecasts within it will be discussed.

S27.2

Effects of stratification variability on the distribution of chlorophyll concentrations and *Pyrodinium bahamense* var. *compressum* in Sorsogon Bay, Philippines

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Sorsogon Bay, located in the eastern side of the Philippines, is one of the sites regularly monitored for occurrence of harmful algal blooms particularly *Pyrodinium bahamense* var. *compressum* (Pbc). Temperature, salinity and density data collected from 2009 to 2013 illustrate that apart from the tidal and wind driven circulation, a stratified estuarine flow specifically a thermohaline (density-driven) circulation cell was also observed in the bay. This was characterized by a net inflow in the bottom layer, net outflow in the upper layer, and upward entrainment along the interface between these two layers. The highly stratified conditions were found in the eastern shallow side of the Bay and a transition zone was observed roughly near the center part of the bay. Further analysis of the transition zone showed that chlorophyll concentrations were observed to be higher at the near bottom part of the water column in all of the sampling duration. This suggests that stratification plays an important role on the distribution of phytoplankton cells. Thus, a spatial statistical model was developed to project Pbc distribution based on in-situ environmental parameters during periods where there were constraints in acquiring actual in-situ Pbc data. The model can then be a tool to assist in the early prediction or detection of Pbc in future monitoring of Sorsogon Bay.

S27.3

CSLAP: New York's citizen-based monitoring program for lake assessment and harmful algal blooms

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In 1985, New York State Department of Environmental Conservation and the New York Federation of Lake Associations established a cooperative citizens-based monitoring program to monitor and track lake water quality throughout New York State. Starting with 25 lakes and 100 volunteers, this Citizen State-wide Lake Assessment Program (CSLAP) now includes more than 230 lakes and 1,500 volunteers. Volunteers are trained in sampling techniques and samples are collected monthly between June and October. On-site measurements include water clarity and temperature. Additional samples are collected for certified laboratory measurements of nutrients (N,P), pH and algal pigments. Starting in 2011, this program was expanded to include the cyanobacteria and their toxins microcystins, anatoxin-a and cylindrospermopsins. Bloom and open water samples collected by citizen volunteers are quickly analyzed using a BBE FluoroProbe to determine cyanobacteria abundance. Samples are then extracted and analyzed for toxins using a combination of tools including PPIA, LCMS and LC-MS/MS. Presence- absence information is then posted on the New York State DEC website. The program analyzes about 1000 samples per year. In addition to providing a rapid monitoring and alert protocol for the protection of lake users against CyanoHABs, information from the CSLAP program is being used to meet Federal Requirements for Lake Assessment and develop state-wide nutrient criteria that can be used as guidance for lake remediation and protection. Cyanotoxin monitoring has allowed us to develop simple and protocols for sample triage and determine those cyanotoxin of most concern in New York State.

S27.4

Implementation of analytical methods to monitor shellfish biotoxins in Australia

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Shellfish biotoxins which are produced by microalgae can be accumulated in shellfish and cause severe human poisoning after consumption of the contaminated seafood. Since 2012 Australian shellfish quality assurance programme adopted the faster and more sensitive chemical analysis methods to substitute mouse bioassay for regulatory testing of amnesic, diarrheic, and paralytic shellfish toxins (ASP, DSP and PSP). Soon after implementation at our laboratory major harmful algal blooms of the toxic dinoflagellates *Alexandrium tamarense* and *Gymnodinium catenatum* attacked the prime shellfish growing area of Australia. The extremely high cell concentrations were observed in some sites and the PSP levels observed in blue mussels (*Mytilus galloprovincialis*) and pacific oysters *(Crassostrea gigas)* exceeded the regulatory limit of 0.8 mg kg^{-1} saxitoxin equivalent in shellfish meat, resulting in enforced closures of commercial shellfish harvesting areas. Analysis of over 3,000 samples and hundreds of positive samples allowed us to better understand the technical performance of the analytical methods and better protect Australian aquaculture industry.

S27.5

A summary of harmful algal bloom monitoring and risk assessment within shellfish harvest areas in New South Wales, Australia

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Since 2005, management of shellfish harvest areas in New South Wales (NSW) has included regular phytoplankton and biotoxin monitoring of 31 estuaries, over 2,000Km of coastline. The data shows harmful algal trends shifting between amnesic shellfish toxin (AST), paralytic shellfish toxin (PST) and diarrhetic shellfish toxin producers over time and between estuaries. As the NSW Food Authority's Shellfish Safety Program has evolved, the option for local harvest programs to utilise more advanced methods for chemical analysis has created an opportunity to compare quantified toxin levels and regulatory limits for phytoplankton cell concentrations. We examined sporadic blooms of *Pseudo-nitzchia* spp., *Alexandrium* spp. and *Dinophysis* spp., comparing Jellet Rapid test kits, high performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis and phytoplankton cell concentrations. Our preliminary data indicate that negative Jellet tests were consistent with detections of ASTs below regulatory limits, as reported by LC-MS/MS. However, during an immense (>6 million cells/L) bloom of *Pseudo-nitzschia cuspidata* in 2010, we observed inconsistent results between chemical methods. We have found, for *Alexandrium catenella* events, that Jellet PST positives have been over-conservative when compared to HPLC analysis. *Dinophysis acuminata* events have also been complex. Cell concentrations above the regulatory limit (1,000 cells/L) do not always correspond with positive biotoxin reports. In these cases, risk assessments have been based on cell concentrations and targeted biotoxin sampling. The continued use of analytical chemical methods will enable improved management of harvest programs and public health risks due to the apparent increase in algal-related toxic episodes.

S28.1

Rapid identification of maitotoxins and ciguatoxins from Caribbean and Pacific *Gambierdiscus* by LC/MS and a new functional bioassay

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Ciguatera is a circumtropical disease produced by polyether sodium channel toxins (ciguatoxins) that enter the marine food chain and accumulate in otherwise edible fish. Ciguatoxins and a potent water-soluble polyether named maitotoxin (MTX) are produced by certain *Gambierdiscus* spp. in the Pacific, but little is known of the potential of Caribbean species to produce these toxins. In this study we established a rapid extraction procedure, based on a modification of the ciguatoxin rapid extraction method (CREM), that facilitated the simultaneous detection of ciguatoxins and maitotoxins from cultures of *Gambierdiscus* by LC/MS and a new functional bioassay. The bioassay used a FLIPR kinetic plate reader to measure changes in intracellular calcium in cultured SH-SY5Y cells assay in response to addition of HPLC fractionated *Gambierdiscus* extracts. Since MTX directly activates calcium influx, changes in intracellular calcium levels are proportional the level of MTX applied. In contrast, CTX activity was estimated from the increase in calcium flux in SH-SY5Y following veratridine addition, a sodium channel activator toxin synergistic with ciguatoxin. Using this approach, we identified significant maitotoxin production in all species tested, including a small maitotoxin in Caribbean species that was previously found in Pacific isolates. In contrast, few *Gambierdiscus* species produced detectable levels of ciguatoxin-like activity. Significant strain dependent differences in ciguatoxin and maitotoxin levels were also identified. The approach outlined can rapidly identify polyether toxins produced by *Gambierdiscus* in culture and has the potential to be applied to field samples collected during blooms.

S28.2

Absolute quantification of Ciguatoxin 1B standard by quantitative Nuclear Magnetic Resonance

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As a part of our effort to prepare reference toxins for use in ciguatera research, we applied quantitative 1H NMR (qNMR) for quantification of ciguatoxin-1B (CTX1B), the representative toxin in the Pacific. Though qNMR is a nondestructive method to give data traceable to the International System of Units, the properties of CTX1B, such as small amounts, multiple protons, and flexible conformation, makes this molecule a challenging target. In this study, we employed an indirect quantification method to avoid contamination from internal standard (IS). Deuterated pyridine-d_5 was used as a diluent of test solution as a volatile IS. The residual protons of pyridine-d_5 were quantified in advance by qNMR using certified reference material. The accurate CTX1B content of the purified sample was determined by the 1H NMR under the following condition; apparatus, 500MHz NMR spectrometer; data acquisition period, 4.0sec; relaxation delay, 60sec; spectral width, 40ppm region; scans, 512 times; pulse angle, 90º; temperature, 5ºC. The obtained spectra were congested with many signals arising from 86 protons residing on the CTX1B molecule and many of them were overlapped with each other. However, the olefinic protons located on carbon 3 and 4 exhibited the highest signal (S/N = 68:1) and good separation from other signals. The quantitative results calculated from these signals indicated 0.135mg and showed good repeatability (RSD, 0.4%). Additional data about the quantification of other CTX analogues using external standards will be presented at the session.

S28.3

Development of a UPLC-MS/MS method for the analysis of toxins produced by *Gambierdiscus*

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A fast, sensitive and selective ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was developed for rapid analysis of ciguatoxins and maitotoxin. The method has been used to screen cultures of *Gambierdiscus* for toxin production and to analyse fish samples. Ciguatoxins predominately form $[M+Na]^+$ adducts when using an acidic mobile phase, these adducts do not fragment meaning only pseudo-MRM (parent to parent) transitions can be used. A novel approach using an alkaline mobile phase increased the abundance of the pseudomolecular ion $[M+H]^+$ over the stable $[M+Na]^+$ adduct. The $[M+H]^+$ ion can be fragmented enabling true MRM transitions to be used instead of previously report pseudo-MRM transitions. It was not possible to monitor a singly charged ion for maitotoxin due to the mass range limitation of the mass spectrometer (<2000 amu). However, with the basic mobile phase a stable di-anion was readily formed making LC-MS analysis of maitotoxin possible. An alternative approach for analysing maitotoxin was also developed. This involved monitoring an oxidative cleavage fragment similar the process recently reported for palytoxin and related analogues.

S29.1

Development and performance of the Phytoxigene™ CyanoDTec Test: A rapid molecular assay for the routine monitoring for the presence of cyanobacterial blooms

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We now have a good understanding of the genetic basis for toxin production by a number of groups of microorganisms. The discovery of these toxin biosynthetic pathways has enabled the development of genetic screening of environmental samples. Molecular genetics underlying *cyanotoxin* production in aquatic environments, but more specifically, the toxin biosynthesis genes have been used to develop a multiplex quantitative PCR assay for the simultaneous detection and quantitation of cyanobacteria 16S rRNA (*16S*), microcystin and nodularin synthetases (*mcy*/*nda*), cylindrospermopsin synthetase (*cyr*) and saxitoxin synthase (*sxt*) genes. Along with the copy number of the relevant *cyanotoxin* biosynthesis gene the internal *cyanobacteria*-specific 16S rDNA control target can be used as a biomass reference target. As part of the development process reference material comprising of plasmid DNA containing defined copy number of target sequences for *16S, mcy*/*nda, cyr* and *sxt* gene was designed and characterised at NMI's laboratories using droplet digital polymerase chain reaction (ddPCR) technology. This reference material was used for the production of standard solutions suitable for constructing calibration curves to be incorporated into the CyanoDTec DNA test kits. Validation and test performance data for the assay will be presented along with details of the development of standards to each of the relevant target toxin genes to which these sequences are quantitated against. This multiplex quantitative PCR assay should become a very important addition to the resources available to laboratories and authorities for better surveillance, detection, prediction and monitoring of harmful algal blooms.

S29.2

Role of dinoflagellate eIF4E family members in translational regulation of gene expression

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Understanding the molecular mechanisms underlying growth and persistence of dinoflagellate blooms is critical for the development of mitigation strategies. The regulation of gene expression in dinoflagellates is distinct from other eukaryotes and a wide range of studies has implicated the recruitment of mRNA for translation as a major site of regulation. However, relatively little is known of the underlying mechanisms. Dinoflagellates have a unique cap-4 structure with variability in the first nucleotide that derives from *trans*-splicing of a 22-nucleotide leader exon from a capped small nuclear RNA. Our studies have focused on a family of cap binding proteins, the eIF4Es, that function in mRNA recruitment and its regulation. Our analysis of transcriptomes from eleven dinoflagellate species has identified multiple eIF4Es in dinoflagellates that fall into three clades. eIF4Es in two of these clades are unique to heterokonts and dinoflagellates, while eIF4Es from the third clade are highly conserved in all alveolates. Using eIF4Es from *Amphidinium carterae*, we have characterized their ability to bind the cap analogue m^7GTP. We have found that the only eIF4E in cell extracts binds m^7GTP is from the most conserved sub-clade of Clade 1. This is in contrast to the m^7GTP–binding ability of recombinant eIF4Es where all Clade I and III eFI4Es bind to m^7GTP, but Clade II eIF4Es do not. This provides the first indication of differential function of eIF4Es in dinoflagellates.

S29.3

Tunicate xenobiotic activated nuclear receptors: their application as sensor elements in high-throughput bioassays for marine microalgal biotoxins

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Xenobiotic activated nuclear receptors (XANRs) regulate transcription of genes involved in metazoan detoxification pathways. The current explosion in genomic sequence data allows detection of putative XANR genes from marine organisms. The filter-feeding Tunicates (phylum Chordata) are of particular interest as they form the sister clade to the Vertebrata. Tunicate XANRs may have adaptively evolved to bind marine bioactive compounds, including microalgal biotoxins. We utilised tunicate XANRs as 'sensor elements' in high-throughput bioassays for microalgal biotoxins. We developed recombinant yeast strains that express chimeric proteins combining tunicate XANR ligand-binding domains (LBDs) along with a generic DNA-binding domain. Such proteins mediate ligand-dependent transcription of a reporter gene (*lacZ*) encoding an enzyme (β-galactosidase) which is easily assayed. Bioassay strains corresponding to XANR LBDs from two tunicates, *Ciona intestinalis* (*Ci*) and *Botryllus schlosseri* (*Bs*), were exposed to both natural and synthetic chemicals (n = 40). Of five microalgal biotoxins tested, three activated both bioassays at nanomolar concentrations: okadaic acid (*Ci*EC_{50} 26 nM, *Bs*EC_{50} 16 nM), pectenotoxin-11 (*Ci*EC_{50} 883 nM, *Bs*EC_{50} 553 nM), and portimine (*Ci*EC_{50} 130 nM, *Bs*EC_{50} 74 nM). Among all agonists, microalgal biotoxins were more potent (i.e. EC_{50} values 2 - 3 orders of magnitude lower) than synthetic compounds, which is consistent with the hypothesis that tunicate XANRs have adaptively evolved to bind commonly encountered natural toxins. Given the large number of tunicate species, occupying a wide range of ecological niches, we propose that tunicate XANRs may act as 'pre-shaped' sensor elements suitable for detection of marine bioactive compounds including microalgal biotoxins.

S30.1

Stoichiometric regulation of phytoplankton toxins

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Global change will have consequences for the availability of important biologically active elements such as carbon (C), nitrogen (N) and phosphorus (P). We investigated to what extent the relative availabilities of these elements regulate the synthesis of C- and N-rich toxins by various phytoplankton groups, including dinoflagellates, cyanobacteria, diatoms and prymnesiophytes. We show that N-limitation causes a reduction in the cellular quota of N-rich toxins, while P-limitation causes an increase in the N-richest paralytic shellfish poisoning toxin. Moreover, we demonstrate that the cellular content of N-rich toxins increases with increasing cellular N:P ratios. These relationships can be explained by changes in synthesis of amino acids, required for building N-rich toxins. Limitation by either N or P promotes the cellular quota, or toxicity, of the investigated C-rich toxins. We thus show that the production of toxins behaves in broadly comparable ways across a wide variety of biota and ecosystems.

S30.2

HABs and phycotoxins in the Northern Benguela current upwelling system: a decade of perspective

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Namibia's Shellfish Sanitation program was established in 2004 to aid the burgeoning bivalve mariculture industry for export purposes. Namibia's coastline is predominantly influenced by the cold Benguela current upwelling system. The upwelled water is nutrient rich aiding in the proliferation of algal blooms, thus providing an ample food source for shellfish mariculture activities. A decade of testing physical, chemical and biological oceanographic parameters has provided valuable insight on algal risk for the region. The occurrence of phycotoxic HABs species has increased over the last five years, with toxic events generally being dominant during late summer and early winter. *G. spinifera* was identified in the region for the first time in 2011, during late summer. LC-MS/MS analysis of field and culture samples revealed a profile dominated by yessotoxin (YTX), homo YTX and new analogue, 22-OH YTX. Molecular phylogeny grouped the Benguela *G. spinifera* with other toxic *G. spinifera* species. PSP values exceeding the regulatory limit have been detected infrequently, however an *Alexandrium* bloom was directly correlated to high PST values in shellfish in 2013. The Receptor Binding Assay produced results comparable with the MBA during this period. In addition, unique to this area is HABs aiding in hydrogen sulphide eruptions in the water column since anoxic water is a consequence of bloom decay. The survival rate of the shellfish tested was greater under low oxygen conditions than under hydrogen sulphide conditions (5-7 days) in laboratory experiments. An effective early warning system can be developed for from this data.

S30.3

Relationship between phytoplankton, microbial community and environmental factors in stratified sediments of a eutrophic lake

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In a eutrophic lake, the sediment is very important for the geochemical cycle of important elements and the cyanobacterial bloom. A 67-cm long sediment core was taken from a site at 17 m water depth using a gravity corer in Daechung Reservoir showing annual cyanobacterial bloom. The subsamples were collected from the core at 1-cm intervals. C_{org}/N_{tot} ratios ranged from 7 to 11, indicating typical of lakes in which the organic matter was from mostly autochthonous algae (mainly planktonic organisms with 6 to 9 of C/N ratio). Total P showed a fluctuation from 104 to 259 μg/g dry sediment which showed a decreasing pattern along a depth of sediment. Notably, several evidences including C/S ratios, dark sediment colour and gas-escaped structures revealed that the lake sediments were under anoxic condition, especially during winter deposition, probably resulting in sulfate reduction and pyrite formation. Diatom assemblages (especially *Cyclotella* spp.) clearly showed that the trophic nature of the lake shifted to the oligotrophic condition after the summer monsoon episode. From sequence-based microbial community analyses, *Proteobacteria* (66.6%) was the most prevalent phylum in all sediment samples, followed by *Chloroflexi* (8.9%), *Bacteroidetes* (5.1%), *Spirochaetes* (2.6%), etc. In most sediments, *Sulfuricurvum*, *Sideroxydans*, *Gallionella*, *Polaromonas* and *Albidiferax*, represented approximately 50% of the bacteria present. In addition, relationship between phytoplankton, microbial community and environmental factors in stratified sediments are under the analysis with an artificial neural network (ANN) system. Consequently, this study can provide information on the geochemical circulation of nutrients by diverse microbial activities in the sediment.

S31.1

A global strategy to advance the science and management of ciguatera fish poisoning

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Ciguatera Fish Poisoning (CFP) is the most extensive human illness caused by harmful algae. Tens of thousands may be affected annually based on estimates that consider the substantial under-reporting of cases. The inability to conduct appropriate tests for the causative toxins leads to extensive closures of fisheries and access to important food resources. Regions beyond those directly experiencing CFP are indirectly at risk via world-wide commerce in seafood. At the 27th Session of the Intergovernmental Oceanographic Commission (IOC) Assembly in 2013, a Recommendation[1] of its Intergovernmental Panel on Harmful Algal Blooms (IPHAB) was adopted and called for the establishment of a global Coordinated Ciguatera Strategy involving IOC-FAO-WHO and others agencies such as IAEA, regional bodies, individual nations and the expertise of ecologists, toxin chemists and medical researchers. This Strategy will include a focus on improving organism detection, sampling strategies, detection of multiple toxins, and epidemiological data collection, reporting and assessments. The goal of the Strategy is to accelerate scientific progress to meet the most pressing societal needs through greater regional cooperation. In order to meet this challenge, strong networks, building on existing mechanisms, are needed to enhance the collaborative efforts already making considerable progress. Community-supported summaries from GEOHAB Benthic Systems[2] and a white paper written for the 11th Session of IPHAB[3] provide strong foundations from which to build the Strategy. This presentation aims to raise awareness about the Strategy and provide contacts to allow individuals and institutions to join this effort at an early stage.

S31.2

Integrated study of a fish poisoning outbreak in Rapa (Australes, French Polynesia)

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Ciguatera had not been described in Rapa, an island located in the southernmost part of the Australes archipelago (French Polynesia), until a 2009 outbreak which affected half the population following a community fishing party in the reservation zone. The integrated study conducted in 2010 examined: i) the epidemiology of this major outbreak; ii) the abundance of CFP-related dinoflagellates in 36 sampling locations; iii) the frequency of ciguatoxicity in fish species (herbivores and carnivores) most frequently consumed locally; iv) the potential presence of a wide range of micro-algal toxins in the environment using the SPATT method. Clinical records indicated symptoms similar to ciguatera in affected individuals with the presence of CTXs confirmed in blood samples of 2 patients. The unusual severity of this outbreak eventually resulted in 2 fatalities. Macro-algal samples confirmed the presence of toxic *Gambierdiscus* assemblages in Rapa and also outlined the potential health hazard associated with *Ostreopsis* mass-proliferation on *Sargassum* sp. and *Lobophora variegata*. Overall, 77% of the 250 fish specimens were found positive to CTXs using the RBA assay. Toxicity varied between species and between locations. Results of ADN LC-MS/MS analysis of SPATT filters indicated the presence of free okadaic acid at levels exceeding the LOR and traces of domoic acid and azaspiracide-2 in Rapa waters. However, there was no clear evidence of these toxins accumulation up the foodweb. Analysis of 2011-2012 epidemiological data showed that education and public outreach actions conducted locally resulted in a decrease in incidence rates attributable to self-regulating behavior among the population.

S31.3

Ciguatoxins in sharks – overview of a recent state of knowledge carried out by ANSES (the French Food Safety Agency)

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In December 2013, ANSES (the French Agency for Food, Environmental and Occupational Health & Safety) set up a Working Group to provide the current state of knowledge about the contamination of sharks, especially tiger sharks (*Galeocerdo cuvier*) and bull sharks (*Carcharhinus leucas*), by ciguatoxins regarding occurrence, analytical methods, human outbreaks and ethology of the sharks. The WG carried out a literature search on human outbreaks associated with the consumption of shark from 1873 to the most recent cases reported in November 2013 and February 2014 in Madagascar (Indian Ocean). After a review of the analytical methods available (*in vivo* mouse bioassay (MBA), *in vitro* cell based assay (CBA), competitive receptor binding assay (RBA), immunological tests, physicochemical analysis), the WG highlighted their strengths and weaknesses for reliable ciguatoxin detection in sharks with regards to the complexity and variability of this toxin family. To better characterize the level of contamination of shark meat by ciguatoxins, the WG suggests a combination of biological and physicochemical methods (e.g. MBA, neuro-2a CBA or RBA and, in case of positive results, LC-MS/MS to confirm the presence of known toxins). A research program has also been launched in spring 2014 to analyse samples of sharks i) from La Reunion (a French island in Indian Ocean) by neuro-2a CBA (MBA results already available) and ii) from Madagascar (samples of the shark associated with a human outbreak in November 2013) by MBA and neuro-2a CBA. Conclusions of the WG and results of this research program will be presented.

S31.4

Giant clams: could they represent a new vector of Ciguatera?

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Ciguatera Fish Poisoning (CFP) is a form of ichtyosarcotoxism caused by the consumption of tropical coral reef fishes that have accumulated ciguatoxins (CTXs), neurotoxins produced by dinoflagellates of the genus *Gambierdiscus*. However, giant clams, frequently consumed in the South Pacific, are sometimes also involved in strong and atypical Ciguatera incidents, as observed in French Polynesia, New Caledonia and Vanuatu. To evaluate the ability of giant clams to accumulate CTXs in their tissues, *in vitro* contamination experiments were conducted. When fed 10 times per day for up 2 days with 2,000 cells.l^{-1} (for a total of 40,000 cells.l^{-1}) of a highly toxic strain of *Gambierdiscus polynesiensis* (containing 7.5 eqv pg P-CTX-3C.cell^{-1}), giant clams appeared toxic by cell based assays (CBA), with a mean of 11.23 eqv ng P-CTX-3C.g^{-1} wet weight. However, giant clams fed with the same concentration of a non toxic strain of *Gambierdiscus toxicus* were found non toxic on Neuro-2A cells. When giant clams were fed with lysed cells of *G. polynesiensis* or *G. toxicus*, similar results were obtained using CBA. These results will be completed by liquid chromatography mass spectrometry (LC-MS/MS) analysis in order to confirm the identity of the toxins accumulated. These first observations indicate that giant clams are able to fix CTXs and could thus constitute another pathway in the ciguatera food chain. This study should contribute to the improvement of seafood poisoning risk assessment and management programs in ciguatera-prone coral reef regions worldwide.

S31.5

Ciguatoxin concentrations in Caribbean lionfish

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With few exceptions, little is known about ciguatoxins (CTX) in Caribbean fishes or how CTX concentrations vary in different fish species. This study measured CTX in 163 lionfish collected from 11 locations throughout the Caribbean and off North Carolina. Lionfish were selected for this study because they are an invasive species causing widespread damage to reef ecosystems and Caribbean resource managers are interested in developing a fishery that could be used as an effective control strategy. However, a safe and effective control strategy depends on whether lionfish are more or less frequently toxic than other reef fish that are consumed by Caribbean subsistence fishers or harvested for commercial markets. This study adapted the fluorescent receptor binding assay (F-RBA) for brevetoxins developed by McCall et al. (2012) to measure CTX levels in lionfish. This adaptation works because both brevetoxins and ciguatoxins activate voltage-sensitive sodium channels in cell membranes. N2a cytotoxicity assays were also run for comparative purposes. Only 10% of the lionfish in this study were found to contain toxin levels above the FDA recommended limit of 0.1 ppb (Caribbean C-CTX-1 equivalents) and only three fish contained >0.3 ppb C-CTX-1 equivalents when measured using the F-RBA. In some locations lionfish tested contained no measurable CTX. The next steps will be to compare CTX levels in a wide variety of Caribbean reef fish and pelagic species to determine if they are more or less frequently toxic than lionfish from the same region.

S32.1

Semi-quantification of microcystins in Alberta lake water

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Microcystins (MCs) are a group of heptapeptides produced by the cyanobacteria. They are transported through the cell membrane by the organic anion transporting polypeptides (OATPs), and inhibit the protein phosphatase 1 and 2A. Exposure to high doses of MCs causes liver failure and death. Low dose is linked to the promotion of liver tumors. Since MCs-producing species are prevalent in Alberta lakes, it is important to monitor toxin levels to protect human and animal health. A cytotoxicity method based on the xCELLigence® system was developed in the ACFT to detect MCs in Alberta lake water. A genetically engineered CHO cell line expressing OATP1B3 (CHO/OATP1B3) was used to detect MCs at sub µg/L concentrations. The real-time response was calculated to semi-quantify total MCs by interpolation from the calibration curve of MC-LR. The method was compared with the protein phosphatase inhibition assay, the enzyme-linked immunosorbent assay and the liquid chromatography tandem mass spectrometry, based on 241 water samples collected from Alberta lakes during the open water season. A good correlation was observed with all methods (*p-value* < 0.0001) at the Canadian recreational water quality guideline concentration of 20 µg/L.

S32.2

Polyacrylamide gel filtration of paralytic shellfish toxins from mass culture of *Pyrodinium bahamense var. compressum* and *Alexandrium catenella* for HPLC laboratory reference material

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Due to low sensitivity and ethical concerns of mouse bioassay in screening and detection of Paralytic Shellfish Toxins (PSTs) in shellfish, Association of Official Analytical Chemists (AOAC) adopted the pre-column oxidation high-performance liquid chromatography coupled to fluorescence detection (HPLC-FLD) as official method in measuring shellfish toxicity, which can quantify individual toxin present in shellfish and prevent animal testing. The method, however, requires standard reference materials which are expensive and not readily available in many countries. Thus we investigate the feasibility of polyacrylamide gel filtration in isolation and purification of PST from mass culture of *Pyrodinium bahamense* var. *compressum* and *Alexandrium catenella*. The goal is to produce suitable reference material for HPLC analysis with gel permeation chromatography using polyacrylamide gel. Chromatographic peaks in each fraction were assessed by comparing it from peaks generated by Certified Reference Material purchased from National Research Council (NRC) - Canada. Suitability of isolated toxin for HPLC was determined by the presence of prominent single peak. Our optimized chromatography set-up has effectively separated PST analogues (Saxitoxin, NeoSaxitoxin, dcSaxitoxin, Gonyautoxin1,4, and Gonyautoxin2,3) to different individual fractions without significant co-elution of unwanted peaks. Samples were subjected to mouse bioassay for confirmation of the result. The volume of toxin produced can provide enough reference material for approximately a year of continuous monitoring of shellfish toxicity. The method was found to be the most efficient way of PST isolation among other procedures because it excludes further purification procedure such as Ion-exchange Chromatography.

S32.3

Partial synthesis of acyl ester analogs of lipophilic marine toxins with analytical and toxicological applications

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Esterification is one of the most important metabolization routes of lipophilic marine toxins in shellfish. In this work we assessed several chemical acylation routes aimed at obtaining acyl ester metabolites *via* partial synthesis from the free toxins. Acylation systems involved both anhydrous and non-anhydrous fatty acid or acid halides as a source of the acyl group; and 4-(dimethylamino)pyridine (DMAP), 1-methyl-2-pyrrolidinone, *N,N'*-dicyclohexylcarbodiimide, or 1,1-carbonildiimidazol as catalyzers of the reaction. Alkaline reaction conditions were provided by pyridine or triethylamine. As a result, we could obtain acyl esters of okadaic acid and dinophysistoxins, gymnodimine-A (GYM-A) and 13-desmethyl spirolide-C, pectenotoxin-2 and azaspiracid-1. The identity of the semi-synthetic acyl analogs was unambiguously confirmed with liquid chromatography coupled to mass spectrometry (LC-MS/MS) by acquisition of the full product ion spectra and the elucidation of fragmentation pathways. Characterization of retention times allowed setting of sensitive and selective LC-MS/MS analysis by monitoring of selected precursor-to-product transitions. Reaction yield depending on reaction time and temperature was tested at sub-nmol scale for the acylation system consisting on palmitic anhydride and DMAP in anhydrous pyridine, showing the best conditions at 75 ºC for 60 min, 75ºC for 120 min and 100ºC for 270 min for cyclic imines, azaspiracid-1 and pectenotoxin-2, respectively. The esterification approach was verified at a larger scale of few mg for esterification of GYM-A, which kept a good yield >90% for the synthesis of 10-*O*-palmitoyl-GYM-A.

S32.4

Food safety testing using the multielectrode array to detect marine neurotoxins

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Detection of marine neurotoxins in seafood products is currently based on in vivo assays and chemical methods. The in vivo mouse bioassay requires the use of a large number of experimental animals and gives a high rate of false positive and negative results while chemical analyses do not allow for the detection of unknown compounds and stand in need of pure standards that are barely available. There is an urgent need for a more animal friendly in vitro test. The present study investigated the suitability of the multielectrode array as a mode of action-based alternative for the screening of marine neurotoxins in seafood. Nine neurotoxic model compounds with similar modes of action as marine neurotoxins, seven pure marine neurotoxins and two contaminated extracts were tested in rat cortical neurons, using neuronal activity as a final readout. Among the nine model compounds a Na^+ channel blocker (diphenhydramine) and opener (veratridine), two Ca^{2+} channel blockers (isradipine, verapamil), two Na^+/K^+ ATPase pump blockers (digoxin, ouabain) and one K^+ channel blocker (amiodarone) induced suppression of the neuronal activity while two K^+ channel blockers (clofilium, sematilide) had no effect on the neuronal activity. Pure brevetoxin-3, domoic acid, pacific ciguatoxin-1, palytoxin, saxitoxin and tetrodotoxin as well as saxitoxin and tetrodotoxin present in two contaminated extracts were successfully detected. Altogether, the data show that the multi-electrode array represents a promising model for the screening of marine neurotoxins. This major finding will not only contribute to the reduction of animal experiments but also improves ensuring food safety.

S32.5

Development of a novel ligand with potential use in radioassay for the monitoring of paralytic shellfish poisoning

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The recent performance of Receptor Binding Assay (RBA) using ^{3}H-STX has allowed its acceptance by the AOAC as an official method of analysis for PSP. Here, we describe the development and evaluation of a novel analog of mu-conotoxin GIIIA as ligand in RBA. GIIIA is a peptide, from a marine snail, with a pharmacologic action similar to STXs. GIIIA was modified by substituting a glutamine to tyrosine (Q18Y). The analog was found to preferentially fold under standard oxidation producing one major fraction that induced paralysis *in vivo*. The structure of the bioactive isomer was confirmed by amino acid analysis and mass spectrometry. Iodination using the chloramine-T method produced mono-iodinated peptide (MIP) and di-iodinated peptide (DIP) derivatives as characterized by LC-UV analysis. The proportion of each labeled derivative was influenced by the relative amounts of peptide and iodide in the reaction. Computational modeling predicted little conformational variation between the native and analog, but the iodine could create steric and electrostatic constraints on the toxin molecule. The binding to rat skeletal muscle Na channel of the analog and its labeled derivatives in competition with ^{3}H-STX exhibited a concentration-dependent inhibition of STX binding, albeit a progressively reduced receptor affinity relative to STX. Labeling with radioiodine produced MIP and DIP with high radiochemical purity but lower specific radioactivity compared to ^{3}H-STX. Evaluating the performance of MIP in a competition assay has demonstrated its potential application as radioligand for a semi-quantitative 'go-or-no go' test to detect STX with sensitivity comparable to the current RBA method.

S33.1

Alexandrium affine, *A. catenella*, *Karenia papilionaceae*, *K. mikimotoi*, *Heterosigma akashiwo*, and *Chattonella* spp. differentially affect the early-life development of Japanese pearl oyster

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The mass mortalities and acute physiological alterations induced by harmful algal blooms (HABs) in bivalves have been reported since the late 1990s. However, their chronic and sublethal impacts are still not well addressed, especially those associated with exposure(s) to HABs known as ichtyotoxic otherwise toxic only to mammals. To shed more light on the interactions HAB/bivalves, we assessed the effects of seven such species of HABs on the early-life development of a representative bivalve species of economic importance, the Japanese pearl oyster *Pinctada fucata martensii*. In a series of laboratory experiments, we exposed fertilized eggs, 3-hours old D-larvae, and 21-days-old larvae of pearl oysters to several densities of seven species of cosmopolitan, bloom-forming dinoflagellates and raphidophytes, namely *Alexandium catenella* (PSTs-producer), *A. affine* (non-toxic, potentially harmful), *Karenia papilionacea* (bloom-forming, considered non-harmful), and *K. mikimotoi*, *Heterosigma akashiwo*, *Chattonella marina*, and *Chattonella antiqua* (invertebrate- and fish-killers). The blooms of the seven species of microalgae are coincident with the spawning season of the Japanese pearl oyster in Japan. The cell densities for each species were set well below the decadal maximal blooming densities reported in the field. All seven species of HABs exerted very mild to severe deleterious effects on embryo development, egg hatching success, and survival and motility of both larval stages. The role played by the different toxins and allelochemicals in the differential inimical effects, and the physical activation of the harmful effects of the raphidophytes will be presented and discussed. An overview of our related up-coming research will be also given.

S33.2

Using genetic markers to determine the effect of seeding on the distribution of a saxitoxin-resistant mutation in softshell clams (*Mya arenaria* L.)

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Some softshell clams have a naturally-occurring genetic mutation of the voltage-gated Na^+ channel that grants them resistance to paralytic shellfish toxins (PSTs) produced by *Alexandrium* spp. blooms. Resistant (R) clams are protected from adverse effects of PSTs and continue to feed during *Alexandrium* blooms, increasing toxin biomagnification. This results in an increased risk of paralytic shellfish poisoning for humans, and requires longer harvesting closures. Historically exposed populations have more R clams than those in other areas, however R clams may pay a cost through slower growth, and can be outcompeted by wild-type clams under non-bloom conditions. Clam seeding has introduced R clams into areas where *Alexandrium* blooms are rare, but those populations appear to revert over time to having fewer R clams. The influence of seeding is the missing piece in our ability to predict the effect of *Alexandrium* blooms on the prevalence of the resistant mutation, and its effect on the ecosystem. This work utilizes genetic markers to determine whether patterns of differentiation at the mutation site and independent neutral markers are indicative of selection for PST resistance in clam populations from the Gulf of Maine. Genetic profiles from clams in four previously identified population clusters were used and demonstrate allele transport in the study area. Clams from the Downeast Institute, which provides seed to municipalities throughout the northeastern USA were also included in this study. This work has implications for shellfish management, as well as modelling evolution and natural selection, especially changes that occur over short timescales.

S33.3

Harmful algal blooms and their impacts on bivalve molluscs

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Harmful algal blooms (HABs) are increasingly recognized as having profound effects upon ecology of coastal seas and upon economics of fisheries and aquaculture. Further, HABs are included in a list of concerns about changes in marine ecosystems that increase impacts of diseases and parasites on important resource species and the food webs that support them. Interactions between harmful algae and bivalve molluscs are complex, species specific and can have major impacts on the interactions of molluscs with their environment. Physiological responses of marine organisms to HABs vary according to the algal/organism combination, but can affect bivalve molluscs at various levels of integration: molecular, cellular, tissular, behavioral, reproductive, etc. In addition, combined effects with pathogens, pollutants or other environmental stressors can modify the physiological responses of bivalve molluscs to HABs and their toxin accumulation. Conversely, an exposure to HABs can change the susceptibility of bivalve molluscs to diseases or to environmental perturbations, thus highlighting the importance of considering multiple environmental factors. This presentation wishes to highlight the direct and indirect impacts of HABs on physiological responses of bivalve mollusc populations, which can clearly have profound effects upon ecology of coastal seas and upon economics of fisheries and aquaculture.

S33.4

Age-dependent changes in toxicity of *Perna viridis* mussels exposed to natural populations of *Pyrodinium bahamanse* var. *compressum*

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The accumulation of saxitoxin in mussel, *Perna viridis*, as a function of mussel age and/or mussel size at high and low cell densities of *Pyrodinium bahamense* var. *compressum* (PbC) is presented. Toxicity in mussels at Boton Site, Sorsogon Bay, Sorsogon, Philippines were assessed weekly within a 130-day sampling period using Receptor Binding Assay (RBA). The site experienced one (1) significant *Pyrodinium bahamense* var. *compressum* (PbC) bloom wherein increase in toxicity was immediate upon increase in PbC cell density. Maximum PbC cell density was attained at 4280 cells/L. At this cell density, mussel toxicity linearly increases with mussel age, with older mussels forty times more toxic than the mussels a year old younger. In terms of weight, this shows an increase of nine units in mussel toxicity per increase in the weight of mussel meat (in grams). A relatively low cell density bloom attaining a maximum of 800 cells/L succeeded this major bloom. However, at this lower cell density, younger mussels are more toxic than older ones. This may correspond to size-specific ingestion rates wherein younger and smaller mussels consume algal cells faster than older and larger mussels. These results showed that resulting mussel toxicity is a function of both age and cell density. At high cell density (~4000 cells/L), specific toxicity increases with age while at low cell density (~800 cells/L), specific toxicity decreases with age.

S33.5

Toxicity assessment of marine dinoflagellates using echinoid fertilization and development, and fish embryo toxicity bioassays

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Toxic marine micro-algae that form harmful algal blooms can have serious human health impacts worldwide. New species are rapidly being described and their associated toxins isolated and characterised. A high level of within-species variation calls for specific testing and examination of isolates from different regions. The benthic/epiphytic dinoflagellate genera *Ostreopsis* and *Gambierdiscus* produce some of the most toxic natural known compounds, including palytoxin, ciguatoxin, and maitotoxin (and associated analogues). These toxins are associated with incurable human syndromes such as ciguatera fish poisoning through consumption of contaminated finfish. The aim of the study was to assess the toxicity of known marine biotoxins (as determined by LC-MS/MS analysis) using two bioassay models. Extracts of 12 *Ostreopsis* and *Gambierdiscus* isolates (originating from the Cook Islands, Noumea, Australia and New Zealand) were characterised using LC-MS/MS methods to determine toxin content. The extracts were tested in the sea urchin (*Evechinus chloriticus*) fertilisation and developmental assay and the zebra fish (*Danio rerio*) fish embryo toxicity (FET) bioassays. Urchin fertilisation was not significantly affected by the presence of any of the algal extracts. Urchin larval development was significantly affected by two of the five *Gambierdiscus* extracts tested and partially affected by three of the seven *Ostreopsis* extracts. The FET assay showed a toxic response to four out of five *Gambierdiscus* extracts with no response to any *Ostreopsis* extracts. These results show the potential of these two bioassays as screening tools for marine algal biotoxins.

S34.1

Ecophysiology of New Zealand *Didymosphenia geminata* nuisance diatom mats, compared to Tasmanian Gomphonema hydrofouling diatoms

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The nuisance value and negative impacts from New Zealand *Didymosphenia geminata* diatom fouling is caused by the massive production of persistent extracellular stalks. As such it compares with nuisance fouling by the smaller stalk-forming *Gomphonema tarraleahae* in Tasmanian hydrocanals. Comparative nutrient and light-adaptation responses were characterised by *in-situ* Pulse Amplitude Modulated (PAM) fluorometry on Buller River (NZ) *D. geminata* and Tasmanian *Gomphonema*. *Didymosphenia geminata* appeared remarkably tolerant towards high light conditions and variable flow regimes, but unexpectedly inhibited by silica, and stimulated by iron. By contrast, *Gomphonema* was inhibited by high light, required high flow and was less sensitive to nutrients. Due to its broad environmental tolerance, accidentaly introduced *Didymosphenia geminata* would be likely to establish in Tasmanian hydrocanals, which exhibit similar conditions to those of New Zealand water courses.

S34.2

Is in-stream hydraulic habitat modelling a useful tool for predicting *Phormidium* habitat and establishment?

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Benthic mat forming cyanobacteria of the genus *Phormidium* are abundant in many New Zealand rivers. When conditions are optimal *Phormidium* can proliferate, forming extensive mats across large expanses of river substrate. This is of considerable concern in rivers that are used as drinking water sources or for recreational activities as *Phormidium* can produce a range of neurotoxic compounds. River flow is commonly cited as the most important physical variable regulating *Phormidium* abundance. However, there have been no in-depth investigations exploring this relationship. To protect the in-stream value of riverine systems, water managers have used hydraulic habitat models to set environmental flow limits. Hydraulic habitat models describe the relationship between river flow and aquatic habitats. In this study over 700 measurements of *Phormidium* habitat preferences (depth, velocity and substrate type) were taken at seven sites along the Hutt River (Wellington). These data were then used to create habitat suitability criteria for the seven sites and applied to three existing hydraulic modelled sites to simulate *Phormidium* habitat availability at varying flows. *Phormidium* habitat suitability was highest on large substrate most likely due to increased substrate stability and surface heterogeneity (which provides refuges for cells during flushing events). The optimal river velocity was found to be less than 1.1 m s^{-1}, this coincides with when minimal shear stress would occur. No depth preferences were measured in this study. When flow reductions were modelled only minor increases in the proportion of available habitat were observed, demonstrating that *Phormidium* can grow in a wide range of physical habitats.

S34.3

Application of a spectrofluorimetric tool (Benthotorch) for the monitoring in rivers of benthic biofilms including potentially toxic cyanobacteria

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Microbial benthic communities in rivers can be dominated by filamentous cyanobacteria but the environmental conditions that favour their development are still unknown. During the last years, numerous cases of animal poisoning due to cyanotoxins produced by benthic cyanobacterial (mainly *Phormidium* sp.) have been described worldwide, which requires the implementation of monitoring programs to limit sanitary risks for humans and animals. Searching for field tools allowing a rapid estimation of the global composition and biofilms biomasses, we test performance of the Benthotorch, which allows quantification, per surface unit, of the biomasses of green algae, diatoms and cyanobacteria. This quantification is based on the relative fluorescence intensity of chlorophyll-*a* following sequential light excitation by diodes emitting at different wavelengths. By the comparison of the results providing by the Benthotorch with those resulting with microscopic and spectrophotometric evaluation, our results indicated that the Benthotorch performed well at the early stages in the development of benthic communities, i.e. with biomasses <5µg eq. chl-a cm^{-2}. On the other hand, when cyanobacteria become dominant in the biofilm and when more important biomasses are reached, the efficiency of the Benthotorch declines. This decrease in the efficiency of the Benthotorch when cyanobcteria are dominant might be explained by two hypotheses: (1) an overestimation of the diatom content, possibly because of their physical positioning in the biofilms and/or (2) an underestimation of *Phormidium* sp. due to its particular pigment composition, which requires another calibration of the tool. Works are in progress to test these two hypotheses.

S34.4

Phormidium blooms in the Hutt River, New Zealand: Identifying nutrient sources

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The Hutt River is one of the most popular rivers for recreational use in New Zealand. However, in recent years risk to recreational users from blooms of the toxic mat-forming cyanobacterium *Phormidium* has increased. Nutrients, in particular the relative concentrations of nitrogen and phosphorus, have been identified as an important factor driving the occurrence of *Phormidium* blooms. Monitoring along the Hutt River indicates that dissolved reactive phosphorus (DRP) concentrations are low along the entire length while dissolved inorganic nitrogen (DIN) increases from low concentrations in the upper reaches of the river to moderate concentrations in the middle and lower reaches. *Phormidium* substrate cover data suggest that blooms are more frequent where DIN concentrations are highest prompting Greater Wellington Regional Council to investigate nitrogen sources within the catchment. Long term monitoring data indicate that the four major tributaries to the Hutt River contribute approximately 60% of the total nitrogen load in the Hutt River. The majority of this load originates from the Mangaroa and Pakuratahi rivers – both of which have significant agricultural land use within their catchments. Concurrent flow gauging results along with nutrient testing of bores, groundwater-fed streams and the river itself indicate that a large proportion of the remaining nitrogen load to the Hutt River comes from inputs of groundwater from shallow aquifers in the middle reaches. These aquifers lie under the urban area of Upper Hutt. Nitrogen inputs from multiple sources as well as the occurrence of blooms at relatively low nutrient concentrations present a significant challenge for future management of *Phormidium* blooms in the Hutt River.

S34.5

Tracking microbial succession and anatoxin production in *Phormidium autumnale*-dominate biofilms

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The prevalence and intensity of toxic *Phormidium autumnale*-dominated benthic blooms in New Zealand rivers has increased. This species produces the potent neurotoxins anatoxin-a and homoanatoxin-a, and consumption of biofilms has resulted in numerous dog deaths. Mechanisms regulating dominance and toxicity of *P. autumnale* are unclear, as these blooms can reach high biomass in low nutrient conditions. Benthic biofilms can harbor a complex community of microbes that can change over time and that may interact to facilitate development and metabolic processing. This work investigates relationships of *P. autumnale* with biofilm microbes throughout succession. Biofilms from three sites in the Hutt River (Wellington) were sampled every two to four days for 32 days following a high flow event. Microscopy and molecular methods were used to identify micro-algal and bacterial biofilm components. Variation in toxin production and proportions of toxic geneotypes were assessed using liquid- chromatography-mass spectrometry and quantitative PCR. A suite of environmental variables were also monitored. Three distinct phases of microbial succession were identified (early, mid and late) in both the micro-algal and bacterial communities. Bacterial composition shifted between these phases several days before the micro-algal community, suggesting bacterial driven succession. *Phormidium autumnale* dominated biofilms after 12 days and grew exponentially despite an influx of diatoms in mid-phase succession. This coincided with the greatest toxins per cell on day 24, and suggests an allelopathic role for anatoxins. Proteobacteria dominated the bacteria community and genera involved in exopolysaccharide production, alkaline phosphatase activity and biopolymer degradation were identified.

S35.1

Ciguatera in Queensland, Australia: case study of a fish poisoning outbreak in Townsville and discussion of the elusive mechanisms of ciguatoxin production

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Ciguatera fish poisoning (CFP) is the most common food poisoning event related to fish consumption in the world. Between Jan 2001-Sept 2012, 285 cases of ciguatera were recorded, with 73% in Queensland (QLD), Australia. This debilitating human neuro-intoxication is caused by herbivorous grazer consumption of benthic toxic dinoflagellates (*Gambierdiscus* spp.), which produce ciguatoxin (CTX) precursors that are modified and bioaccumulated up the food chain. An unprecedented outbreak of 17 CFP cases occurred in Townsville, QLD in March 2014 that was attributed to consumption of Spanish mackerel (*Scomberomorus commerson*). Symptoms associated with the Townsville incident will be discussed. Whilst Spanish mackerel is a frequent culprit of CFP (32% of cases), many other species of fish have been implicated. Platypus Bay, Fraser Island is the only site in QLD known to frequently harbour ciguateric fish with a number of species classified as 'no-take' in this area. A survey of *G. toxicus* in April, 2011 revealed bloom proportion of cells (586 cells.ml^{-1}) in a mid-depth region (8 m). However, ciguatoxin precursors (P-CTX-3B, -3C, -4A, -4B) and maitotoxins (MTX) were not detected. Preliminary findings also reveal CTX fish metabolites (P-CTX-1, -2 and -3) were not present in a range of herbivorous, omnivorous and carnivorous fish, including sharks collected during the bloom. The mechanisms involved and the factors that stimulate CTX production are poorly understood. Non-toxic blooms of *G. toxicus* can occur and the unpredictability and sporadic occurrence of ciguatoxic fish have previously been noted in QLD. The implications of these findings will be discussed.

S35.2

Ciguatera 2.0: A website as a helpful tool to improve ciguatera surveillance, risk management and research advances

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Ciguatera fish poisoning (CFP), associated to consumption of toxic reef fish, represents a significant health and economic concern of seafood dependant populations. Long regarded as a tropical islands concern, the occurrence of CFP cases insidiously increases in areas hitherto spared due to expansion of tourism, international trade and global warming. Therefore, it now appears essential to set up a global and dynamic monitoring system of CFP that could benefit to health framework, general population, fishermen, etc, of every concerned country. For many years, French Polynesia has benefitted from a risk management and epidemiological surveillance program which enables monitoring of CFP evolution throughout its territories. However, this monitoring network, somewhat archaic, is declining and needs to be consolidated. In response, a participatory website dedicated to CFP has been elaborated in order to improve the exhaustiveness of recorded cases (surveillance questionnaire), offer a dynamic cartography of ciguatoxic and safe areas/fishes as well as general information about CFP. It will also be a useful research support by collecting knowledge about health complications of CFP and will propose a communication platform for general users, patients, physicians and scientists. This website has a real potential as an effective risk management tool, a dynamic surveillance implement and support for CFP research. Initially created to reinforce CFP management in French Polynesia, this website could become a referent tool for a worldwide surveillance by contributing to a unique database incremented and accessible to everyone.

S35.3

Gambierdiscus and *Ostreopsis* from Cook Islands and New Zealand and the risk of CFP from New Zealand seafood

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Gambierdiscus and *Ostreopsis* are dinoflagellate genera associated with ciguatera fish poisoning (CFP), an illness which is common in the Cook Islands and may be fatal. *Gambierdicus polynesiensis*, *G. australes* and *G. pacificus* were isolated from samples collected in the Cook Islands (2008-2014). *G. polynesiensis* produced ciguatoxin and the latter species produced maitotoxin (MTX) and/or its analogues; palytoxin was detected in some isolates of *Ostreopsis ovata*. *Gambierdiscus* cf *yasumotoi*, identified from Northland, New Zealand (2013), produces putative MTX-3. A sampling trip in February 2014 failed to detect *Gambierdiscus*, but *O. siamensis* formed dense mats on macroalgae throughout the region. A benthic/epiphytic mesh sampling device was deployed for comparative assessment against standard methods and grazing fish were caught for toxin analysis. The Kermadec Islands are a New Zealand territory 1,100 km to the northeast, with anecdotal reports of CFP. A November 2013 voyage of the research vessel Braveheart to document the region's biodiversity (led by Auckland Museum) resulted in isolates of *Ostreopsis* and *Gambierdiscus* from Raoul Island. *Ostreopsis* was cultured for DNA and palytoxin analyses, but compressed *Gambierdiscus* isolates did not survive. The Kermadecs are a staging point for fish migrations between New Zealand and the tropical Pacific and, with climate changes, more tropical fish can be expected in New Zealand's coastal waters. The current CFP risk is negligible, but *Gambierdiscus* will continue to be monitored and *Ostreopsis siamensis* mats are now an annual event.

S35.4

Evaluation of the effects of irradiance on the growth of the Japanese *Gambierdiscus* species and their vertical distribution in Japanese coastal waters

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We examined the effects of irradiance (0.00–420 µmol photons m^{-2} s^{-1}) on the growth of the *Gambierdiscus australes*, *G. scabrosus*, *Gambierdiscus* sp. type 2, and *Gambierdiscus* sp. type 3 strains isolated from Japanese coasts. All species/types reached maximum growth at irradiances ≤427 µmol photons m^{-2} s^{-1}. They adapted to relatively low light conditions, exhibiting an optimum growth range, I_{opt} ($\mu \geq 0.8 \times \mu_{max}$), at 73–430 µmol photons m^{-2} s^{-1} and required only 10.1–11.4 µmol photons m^{-2} s^{-1} (I_o: lower light limit for growth) to maintain growth. The I_{opt} and I_o respectively represent 3.8–23% and 0.50–0.60% of the ambient irradiance present in the surface waters of the temperate region of the Japanese coast, such as Tosa Bay, Kochi, Japan. The I_{opt} and I_o putatively appear at depths of 13.5– 29.7 m and 46.5–47.6 m, respectively. Next, we investigated the vertical distribution of *Gambierdiscus* spp. in an area of the western part of Kochi Prefecture from January 29, 2013 to November 1, 2013. Samples of macroalgae were collected at two depths (1 and 30 m) from the site using snorkeling and/or SCUBA. Mean abundance of *Gambierdiscus* spp. at the deeper site and that at the shallow site was 3.69 cells g^{-1} fresh weight (fw) algae and 0.05 cells g^{-1} fw algae, respectively. The former exceeded the latter by a factor of 73.8. These data suggest that *Gambierdiscus* spp. may bloom at deeper layers rather than at the surface layer in Japanese coastal environments.

S35.5

Does *Gambierdiscus* really exhibit substrate preferences?

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Ciguatera is a form of seafood poisoning caused when people eat fish that contain ciguatoxins. The fish accumulate ciguatoxins through trophic transfer, the source of the toxins being the benthic dinoflagellate, *Gambierdiscus*. Researchers have studied the abundance and distribution of *Gambierdiscus* cells on various algal substrates, and found that cells were more abundant on some host algae versus others - when standardized to algae wet weights. This approach discounts the importance of algal surface area as a factor influencing cell abundances on host algae as noted in many studies. Natural (algae, seagrass, and sand) and artificial (PVC tiles and screens) substrates were collected over a three-year period in the Florida Keys. *Gambierdiscus* abundances were then computed as a function of surface area, which was determined using image analysis. *Gambierdiscus* abundances were highly correlated (except sand) and were within an order of magnitude on all substrates. These results indicate that 1) *Gambierdiscus* distributions followed similar dynamic changes over time, regardless of substrate; and 2) *Gambierdiscus* cells were just as likely to settle on sand as on algal hosts. The importance of these findings is that *Gambierdiscus* cells may not exhibit substrate preference after all, but may rather reflect their tychopelagic life style, where they are continually dislodged and resettled to the benthos with changing hydrodynamic conditions.

S36.1

Karenia brevisulcata: a threat to New Zealand aquaculture?

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Karenia brevisulcata is a toxic dinoflagellate species that bloomed in Wellington Harbour, New Zealand, during the summer of 1998 and displayed unprecedented toxicity to marine life. Although only a single bloom has been documented to date, research on this HAB species has continued due to its severe affects and potential to devastate aquaculture species should it bloom in a commercially sensitive area. This is heightened by the fact that the Marlborough Sounds, New Zealand's premier aquaculture area, is situated <50 km from where the original HAB event occurred. It is also of concern that major *Karenia spp.* blooms recur on a 4-5 year cycle in temperate areas favoured for aquaculture. Aquaculture remains an important industry to the New Zealand economy contributing over $400M annually, with projections that it will be worth $1B annually by 2025. This presentation will draw together information generated over the past 15+ years on *K. brevisulcata* to determine the magnitude of the threat posed to New Zealand aquaculture. This includes the structural elucidation of numerous polycyclic ether toxins responsible for 'Wellington Harbour Toxin' (brevisulcenal and brevisulcatic acid toxins) and their toxic effects on finfish and invertebrate larvae. The development of an analytical method (LC-MS/MS) to monitor toxin levels in seawater, algal cultures and shellfish will be presented as will the setting of phytoplankton trigger levels for *K. brevisulcata* to ensure the successful management of future blooms.

S36.2

New toxins in the group of emerging toxins: azaspiracids, dinophysistoxins, spirolides and karlotoxins

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So-called emerging toxins comprise a not yet well defined group of lipophilic marine phycotoxins associated with HABs for which the toxic effects in the mouse bioassay, mode of action and/or human health significance remain unclear. Some groups of lipophilic toxins are relatively well investigated, such as the classic diarrhetic shellfish poisoning (DSP) toxins, including okadaic acid and dinophysistoxins produced by several species of *Dinophysis* and *Prorocentrum*, or azaspiracids responsible for azaspiracid shellfish poisoning (AZP) synthesized by a few species of *Azadinium* and *Amphidoma*. Nevertheless, new toxin analogues of these groups are being discovered but where the toxin potency is unknown. Regulatory limits for maximum levels of certain phycotoxins in seafood have been established due to their toxic effects in humans. Yet several classes of phycotoxins, such as spirolides produced by *Alexandrium ostenfeldii* or karlotoxins from *Karlodinium veneficum*, are not currently regulated because of their relatively low oral toxicities and/or to the very limited availability of these compounds for toxicity testing. Here we give evidence on some new compounds of the above mentioned toxin groups; two of them are isomers of the regulated toxins DTX-1 and AZA-1 and thus may be potentially misinterpreted. We also provide mass spectral information of six spirolides and five karlotoxins, which have not been previously reported. This includes spirolides produced by *Alexandrium ostenfeldii* and karlotoxins produced by *Karlodinium veneficum*.

S36.3

Analysis of polycavernoside A in the red alga *Gracilaria edulis* that caused previous fatal food poisonings in Guam and Philippines

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Polycavernoside A (PA), a toxic glycosidic macrolide, was first isolated from the red alga *Gracilaria edulis* as the causative agent of the fatal food poisoning occurred in Guam, 1991.[1] PA was also identified in the same alga which caused similar fatal poisonings occurred in Philippines in 2002 and 2003.[2] In this study, we investigated the contents of PA in *G. edulis* previously collected, following these incidents in Guam (collected with the help of Dr. R. L. Haddock, and Mr. Paul Eilers helped extraction) and Philippines using LC/MS. Among the five lots of *G. edulis* of Guam's case, the contents of PA in three lots were 600, 650, and 34 ng/g, respectively, while other two were less than 20 ng/g. PA in one lot of *G. edulis* of Philippines' case was approximately 100 ng/g. The attachments to these toxic *G. edulis* were washed out with water and then, size-fractionated to quantify PA in each fraction. In Guam's *G. edulis*, approximately 50% of the total PA remained in the washed alga, and the concentrations of PA in the washed alga and the attachments were almost same, whereas in Philippines' *G. edulis*, approximately 90% of the total PA remained in the washed alga. PA was also identified in the size-fractions larger than 53 µm and 0.45-53 µm, prepared from the drip of Guam's *G. edulis*. The cyanobacteria attached to *G. edulis* were phylogenetically analyzed based on the16S rDNA sequences.

S36.4

Effect of environmental and biotic factors on the growth and paralytic toxin profiles in isolates of *Gymnodinium catenatum* from the Pacific coast of Mexico. A review

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Isolates of *Gymnodinium catenatum,* from the Pacific coast of Mexico, were cultivated under different salinities, N:P ratios, and temperatures. *In vitro* studies reveal that *G. catenatum* isolates of this region tolerate a broad salinity range (15 to 40), N:P ratios (5.4 to 74.3), and a wide temperature range (15 to 33° C). Grazing experiments with the copepod *Acartia clausi* and the dinoflagellate *Noctiluca scintillans,* show that both grazers feed actively on the toxic dinoflagellate, suggesting that they play a key role in controlling the population of *G. catenatum*. Additionally, mixed cultures demonstrated that *Chattonella* sp. dominates over *G. catenatum* in less than 72 h, probably due to allelopathic effects. Data suggest that salinity, N:P ratios, and temperature modify growth rate and cell density of *G. catenatum*. Additionally, N:P ratios and temperature cause changes in the toxin profile, particularly the decarbamoyl and sulfocarbamoyl toxins; however, no change in the toxin content in cells were observed. Laboratory experiments established that the isolates tolerate a wide range of environmental conditions with no major changes in the toxin profile, and that *G. catenatum* could be present in the water column year-round. However, biotic factors, such as allelopathy and grazing seem to play an important role in the bloom dynamics of *G. catenatum*, which should not be overlooked.

S36.5

Bottom-up proteomics and homology-driven identification of distinct protein spots among various toxin-level differing *Alexandrium* species

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The genus *Alexandrium* is considered to be a non-model toxic dinoflagellate which causes Paralytic Shellfish Poisoning (PSP) due to saxitoxin and its analogues. The exact biosynthesis pathway of saxitoxin production has not yet been elucidated in dinoflagellates mainly due to its large genomic size. A proteomic analysis thereby sheds light on functional molecules produced by the organism which may play roles in toxin production. This present study investigated proteins generated from comparative analysis of various *Alexandrium* species isolated in Philippine waters which include *Alexandrium tamarense* complex- Bolinao strain, *Alexandrium tamarense* complex- Anda strain, *Alexandrium tamutum* and *Alexandrium affine*. Toxin profiles showed marked differences in toxicity of these dinoflagellates at exponential phase. A tandem bottom-up proteomics revealed significant differences in proteome profiles especially at low molecular weight region (~40 KDa and below) across different species. Distinct and upregulated protein spots (>2 fold) as determined using PDQuest™ software v. 8.0.1 were further analyzed using MALDI-TOF. A subsequent multi-layered bioinformatics using BLAST and EST database analysis for homology-driven protein identification was then employed. De novo peptide sequencing of MS/MS spectra were performed using PEAKS 7 software and were further subjected to MS-BLAST analysis for confirmation and for cross-species identification of low hit results. Results identified possible molecules which may be involved in the saxitoxin production including a bifunctional ornithine acetyltransferase/N-acetylglutmate synthase. This study, being the first of its kind in the Philippines, provides insight on functional proteins which may play various cellular roles including toxin production among different species of *Alexandrium*.

S37.1

Links between open sea and coastal waters in the NW Mediterranean: Deep water formation and *Phaeocystis* sp. foam events

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A monthly study of the nutrient phytoplankton dynamics in the coastal area of Barcelona, carried out between March 2002 and March 2006, revealed the regular occurrence of winter nutrient concentration maxima followed by a winter-early spring phytoplankton bloom dominated by diatoms and nanoflagellates. The main diatom taxa in the peaks of 2002 to 2005 were *Chaetoceros* spp. and *Pseudo-nitzschia* spp. However, in the winter-early spring of 2006, the community was dominated by a bloom of *Phaeocystis* sp., which was associated to a notable deterioration of coastal water quality due to foam accumulation, and the accompanying diatom taxa (mainly *Bacteriastrum delicatulum* and *Rhizosolenia* cf. *imbricata*) were different from previous years. Examination of the hydrographical characteristics of the coastal waters during the fertilization events showed that the main nutrient source for the concentration maxima of 2003 and 2004 was land runoff, while the nutrient enrichment originated from intrusions of offshore intermediate waters in 2005 and of offshore surface waters in 2006. This input of open sea surface waters onto the coast could explain the different composition of the diatom community and the dominance of *Phaeocystis* sp., which is an important component of the winter-spring bloom in offshore waters of the NW Mediterranean. The different fertilization mechanisms in the winters of 2005 and 2006 can be associated to the cold and dry winters of these last two years in the NW Mediterranean, which resulted in a particularly intense formation of deep water, both by cascading and by convection.

S37.2

Winter distributions of *Dinophysis* populations: do they help to predict the onset of the bloom?

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Blooms of diarrhetic toxin (DSP) producers of the genus *Dinophysis* (*D. acuminata, D. acuta*) pose the main threat to the sustainable exploitation of cultivated mussels and other bivalves on the Atlantic coasts of Europe. *Dinophysis* spp. do not seem to rely on cyst-germination as a seeding strategy. Detection and evaluation of holoplanktonic populations surviving after a bloom declines may be the key to predict the initiation of the next year's bloom. Three cruises were carried out on the shelf off the Galician Rías Baixas in February 2013 (DINVER 2013), January 2006 (DINVER 2006) and May-June 1993 (MORENA 93) to explore winter and pre-bloom distributions of *D. acuminata* and *D. acuta*. Sampling protocols were adapted to be able to detect extremely low densities of *Dinophysis* (detection level of 2-3 cells l^{-1}). Retention areas with potential inoculum populations, as previously described in other upwelling regions, were not found on these cruises. Here we make a retrospective analyses of the data from these cruises, and identify common hydrodynamic patterns, and accompanying microplanktonic communities in an attempt to untangle a crucial question in *Dinophysis* population dynamics: where do the inculum populations come from?

S37.3

Recurrent toxic blooms of *Alexandrium* spp. in the East China Sea - potential role of Taiwan Warm Current in bloom initiation

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Large-scale dinoflagellate blooms started to appear in the coastal waters adjacent to the Changjiang River estuary (CRE) in the East China Sea at the beginning of the 21st century. The oceanographic and ecological mechanisms, as well as the impacts of these harmful algal blooms, received much attention over the last decade. During the studies in the coastal waters adjacent to the CRE from 2004 to 2007, recurrent blooms of *Alexandrium* spp. were observed for the first time. The major causative species was identified as *Alexandrium catenella*. Analysis of the samples collected during the blooms of *Alexandrium* spp. revealed the presence of paralytic shellfish toxins dominated by low-potency *N*-sulfocarbamoyl toxins C1 and C2. Toxin content in the *Alexandrium* cells ranged from 17.08 to 33.59 fmol.$cell^{-1}$. Blooms of *Alexandrium* spp. occurred generally in April and May, but the initiation time of the blooms varied from year to year. It's suggested that initiation of the blooms have little connection with nutrients, as other algal blooms co-existed in this area were more significant in terms of scale and intensity. The initiation time of the blooms, however, seems to be more closely related to the seawater temperature at the bottom, which is affected by the intrusion of Taiwan Warm Current (TWC). Patches of the *Alexandrium* blooms were mainly found in the area of 29.0-31.0°N, 122.0-123.0°E, with water depth between 20 m and 50 m, but the distribution of the blooms varied from year to year. The distribution pattern, as well as the cell density of *Alexandrium* spp. during the blooms, was related to the initiation time of the blooms. It was proposed that intrusion of TWC in the sea area adjacent to the CRE may trigger the blooms of *Alexandrium* spp. and affect the distribution and intensity of the blooms. This hypothesis, however, requires more detailed studies on the blooms of *Alexandrium* spp. in this region.

S37.4

Are harmful algal blooms becoming the greatest threat to inland water quality in the United States? Perspectives after a decade studying *Prymnesium parvum*

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Though environmental monitoring, assessment and management programs have been developed for chemical contaminants and other stressors in inland waters, the magnitude, frequency and duration of harmful algal blooms (HABs) may be increasing at the global scale. HABs are caused by complex factors that vary among algal species, but landscape modification, from anthropogenic activities ranging from effluent discharges, natural resource extraction and agricultural runoff to salinization and climate change influences on freshwater ecosystems, appear key forcing factors for HAB development in inland water of the U.S. When these HABs are observed, impairment of aquatic life, recreation, agricultural and potable uses of surface waters soon follows. However, impairments of such uses have not yet resulted in designations of exceedances of water quality criteria or standards for surface water bodies under the U.S. Clean Water Act. For example, in the case of HABs from *Prymnesium parvum* (a.k.a., Golden Algae, or the Texas Tide), devastating fish kills have become so routine that fisheries managers no longer stock fish in affected lakes and reservoirs. In some inland habitats, the prevalence of HABs appears to represent more significant threats to sustainable environmental quality than conventional chemical contamination. In fact, toxins produced by HABs are now considered contaminants of emerging concern by the U.S. Environmental Protection Agency. Unfortunately, conventional approaches to resource assessment and management appear poorly prepared to address this question. With the prospects of environmental quality changes from urbanization and climate change continuing to be understood, such observations have prompted the question, Are HABs becoming the greatest threat to inland water quality? This question is considered through perspectives gained and lessons learned following a decade studying *P. parvum* in inland waters of the U.S.

S38.1

Connections between HABs and coral reef phase shifts: investigating macroalgal growth dynamics

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Alongside coral reef impacts, the incidence of the seafood borne illness, ciguatera, has become more prevalent. As the epiphytic benthic dinoflagellate, *Gambierdiscus*, responsible for this illness resides on various species of macroalgae, it becomes imperative to investigate the ecology of macrophytes. Impacts to coral reefs such as bleaching events and disease outbreaks have been shown to provide new substrate for macroalgae to colonize, often resulting in a reef phase-shift. Implications associated with such phase-shifts could exacerbate ciguatera by providing more habitat for *Gambierdiscus*. Results from a three year study involving monthly sampling of a post phase-shift coral reef has shown these marine habitats to be very dynamic systems. While some macroalgae were stable (e.g., *Halimeda*), others were highly variable (e.g., *Dictyota*). Because ciguatoxins are known to enter the foodweb via herbivory, knowledge of algal abundance and palatability is important when considering the trophic transfer of ciguatoxins. Although chemically defended, *Dictyota* is considered palatable by mesograzers. In contrast, *Halimeda* is considered to have low palatability as it is well defended chemically and structurally. Therefore, dynamic but palatable macroalgae may serve as important vectors of ciguatoxins when their abundances are highest. In addition, highly variable species could influence the feedback loops governing phase-shift dynamics by way of impacts to coral recruitment through changes in bare substrate. Possible connections between coral reef phase shifts and incidence of ciguatera could provide a highly motivating factor towards implementing measures to both, the monitoring of HABs as well as the management and conservation of coral reefs.

S38.2

Assessing ciguatera fish poisoning risk: A two tiered monitoring protocol

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Ciguatera fish poisoning (CFP) is a debilitating human illness common in tropical regions. The ciguatoxins which cause CFP are produced by macrophyte-associated *Gambierdiscus* species. These toxins bioaccumulate and persist in marine food webs, threatening seafood resources, local economies and human health. Increased *Gambierdiscus* abundances are often associated with increased CFP risk. Unfortunately, no standardized monitoring strategy for detecting changes in *Gambierdiscus* abundance or species assemblages exists. This study developed a standardized, cell-based method for assessing CFP risk which utilizes artificial substrate (fiberglass window screen) for collecting cells. *Gambierdiscus* cells were shown to associate with the artificial substrate in direct proportion to their abundance on adjacent macrophytes and to reach equilibrium with the surrounding population within 24 h. No difference was observed in the variability of abundance estimates gathered from natural vs. artificial substrates. The screen method was found to be advantageous because it allows normalized cell densities per square cm of substrate among sites, eliminates macrophyte preference issues and provides material that is cleaner and easier to count. CFP risk assessment can be determined from weekly cell counts, coupled with *Gambierdiscus* species composition data determined by species-specific qPCR assays. Elevated *Gambierdiscus* abundances would trigger surveillance of ciguatoxins in herbivorous and carnivorous fishes using cytotoxicity, receptor binding or LC-MS ciguatoxin measurements. This two tiered monitoring strategy provides a cost-effective means to systematically evaluate specific habitats for CFP risk.

S38.3

Effects of long-term repeated exposure of Pacific ciguatoxins (P-CTXs) in orange-spotted groupers (*Epinephelus coioides*)

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Ciguatoxins (CTXs) are produced by marine benthic dinoflagellates such as *Gambierdiscus* spp., and can enter the coral reef food webs when grazers forage on macroalgae. They are then bioaccumulated and biotransformed in fishes. Carnivores at higher trophic levels are generally exposed to higher levels of CTXs via their diets. A number of laboratory and field studies have shown that CTXs induce mortalities of crustaceans, fishes, and marine mammals. To examine chronic effects of Pacific CTXs (P-CTXs) on predatory fishes upon long-term repeated exposure, P-CTXs (low-dose: 0.1 ng/g, 0.2 ng/g; high-dose: 0.6 ng/g, and 1.5 ng/g) were administrated orally to orange-spotted groupers (*Epinephelus coioides*) for 30 days. The body weight, total length, respiration rate, appetite, swimming pattern and survival of orange-spotted groupers were monitored over the course of P-CTX exposure.
After 30-day P-CTX exposure, no mortality of fishes was observed for the treatment and control groups. Yet, fishes fed with high-dose P-CTXs exhibited negative growth in terms of their total body weight, probably due to reduced appetite. In the high-dose groups, the amount of food consumed by fishes started decreasing on Day 6 and Day 13, respectively. Significant decrease in respiration rates were observed in groupers fed with high-dose P-CTXs diet as compared with those in the control group. Tilted swimming was found in groupers fed with high-dose of P-CTX diet. Fishes displaying abnormal swimming in a coral ecosystem might conceivably become more susceptible to predation. An elimination of P-CTX-sensitive fishes could affect structures, functions and stability of food webs.

S38.4

Shedding light on *Gambierdiscus* community diversity: development of Fluorescence In Situ Hybridization (FISH) probes to detect and enumerate *Gambierdiscus* species

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Ciguatera fish poisoning (CFP) is a syndrome caused by the bioaccumulation of lipophilic ciguatoxins in coral reef fish, and their subsequent consumption by humans. These phycotoxins are produced by *Gambierdiscus*, a tropical epiphytic dinoflagellate genus that lives on many varieties of macroalgae, but also may occur on dead corals and in sand. Globally, tens of thousands of individuals are likely afflicted with CFP on an annual basis, with up to 10% of the local population on some islands in endemic areas becoming ill. The ability to accurately and quickly distinguish *Gambierdiscus* species and determine community composition is central to assessing CFP risk. Prior studies have shown that multiple *Gambierdiscus* species co-occur, and have demonstrated differences in toxicity. In order to investigate the dynamics of *Gambierdiscus* species diversity over time and among locations, tools for species identification are needed. Current methods of quantitative PCR are able to provide this discrimination; however, due to differences in genomic content between cells, these methods are less accurate than microscopic counting in determining cell abundance. Since most *Gambierdiscus* species are indistinguishable using light microscopy, we designed fluorescence in situ hybridization (FISH) probes to differentially label species, thus permitting their enumeration using fluorescence microscopy. This technology enables the assessment of community composition and determination of cell abundances. Probes detecting *G. caribaeus*, *G. belizeanus*, *G. carolineanus*, *G. ribotype I* and *G. carpenteri* were designed using alignments of large subunit ribosomal sequences, and were tested on cultures of *Gambierdiscus,* and using field samples collected from the Florida Keys and St. Thomas, USVI.

S39.1

Analytical methods and reference materials for cyanobacterial toxins

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Cyanobacteria are found in marine and freshwater environments worldwide. Some species produce potent toxins called cyanotoxins that present a serious threat to wild animals, pets, livestock and people. Cyanotoxins include hepatotoxins such as microcystins and cylindrospermopsins and neurotoxins such as anatoxins, saxitoxins and β-N-methylamino-L-alanine (BMAA). The main routes of exposure to humans are through contaminated water (drinking, breathing aerosols and skin contact) and dietary supplements made from algae. Sensitive multi-toxin detection methods are required for the screening and quantitation of cyanotoxins in water and algal samples. This presentation will cover efforts to develop and validate such methods, which are based on either hydrophilic interaction or reversed phase liquid chromatography combined with tandem mass spectrometry. Particular emphasis will be placed on BMAA, which has become quite controversial because of it having been implicated with ALS and reported difficulties with analytical methods. A major problem in this field has been the limited availability of certified reference materials (CRMs) for instrument calibration and method validation. Results to date on feasibility studies for preparing a freeze-dried cyanobacterial RM and future plans for a CRM will also be presented.

S39.2

Turn-key liquid chromatography-mass spectrometry methods for identification and quantitation of marine and freshwater algal biotoxins

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Liquid chromatography-mass spectrometry (LC-MS) has evolved as the technique of choice for the determination of various algal toxins. On-going developments in methodology and instrumentation continue improvements in sensitivity and efficiency of analysis. The technology has now evolved to a point where "turn-key" methods are a reality. Such methods prescribe conditions for sample preparation, specifying LC and MS parameters, and provide pre-constructed libraries of selective reaction monitoring (SRM) transitions and product ion spectra for a range of toxins. Turn-key methods have been developed for marine and freshwater algal toxins on AB SCIEX's mass spectrometry platforms allowing for their simultaneous detection, quantitation and confirmation. For marine toxins, the lipophilic groups and domoic acid are monitored using reversed phase LC, while hydrophilic interaction liquid chromatography is employed for the paralytic shellfish poisoning toxins. Reverse phase LC conditions are used for the freshwater microcystins. The methods allow for quantitative SRM determinations as well as data-dependent acquisition of full product ion spectra for any compounds detected by SRM. Searching acquired spectra against the developed libraries can confirm toxin identity. Summary validation data for each method will be presented along with applications to field samples.

S39.3

Docking of benzoate saxitoxin analogs of *Gymnodinium catenatum* Graham in the voltage-gated sodium channel Na_v 1.4

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Saxitoxin (STX) and its analogs are potent alkaloids with neurotoxic properties. They block the voltage-gated sodium channel pore (Na_v) with a 1:1 affinity. This impedes the flow of Na^+ ions into the cell, preventing action potentials in the peripheral nervous system and skeletal muscles. Some theoretical and experimental models have been proposed for the STX binding mechanism, which involves the two positively charged guanidinum groups and the hydroxyl groups located at carbon 12 and are present in all STX analogs. Our theoretical study employs a Na_v homology model (Na_v 1.4) to perform molecular docking studies with 35 saxitoxin analogs, of which 17 belong to the benzoate-type analogs. These STX analogs have been identified only in *Gymnodinium catenatum* and have not been rigorously studied for lack of standards. Our theoretical results show that the STX analogs bind to the Na_v with equal or higher affinities than STX, the most potent toxin of these analogs. However, the binding affinities (ΔG, kcal/mol) *in silico* do not have a linear relationship with the toxin potency obtained experimentally in previous studies. Our simulation is the first *in silico* modeling study achieved with benzoate-type paralytic toxins on the Na_v pore. This contributes to a better understanding of the theoretical modes of action of the STX analogs.

S39.4

Transfer of isotope-based receptor assay for paralytic shellfish toxins to the end-user: the Philippine setting

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The isotope-based Receptor Binding Assay for Paralytic Shellfish Poisoning (RBA-PSP) was established in the Philippines through a national program in 1998 based on the recommendations of the Expert Mission sent by the International Atomic Energy Agency (IAEA). Under the program, an RBA facility was established and personnel were trained for RBA work. Further studies were also undertaken to determine the advantages of the RBA technique against Mouse Bioassay (MBA) and High-Performance Liquid Chromatography (HPLC) methods. The results of the inter-assay comparison and the in-house validation showed that RBA is a better alternative over other methods, however, it was not immediately adopted by the regulatory body for the following reasons: (1) acceptance of RBA as an official national method of analysis for PSP, (2) considerations on the use of radioactive materials, and (3) logistics and other financial aspects in building up and maintaining the RBA facility. In this paper, the role of international collaboration, the importance of national government support, and the strategies being undertaken to address the issues under the capacity of a developing country like the Philippines are discussed.

S40.1

The effect of pre-industrial and predicted atmospheric CO2 concentrations on the development of populations of the cyanobacteria species, *Anabaena circinalis* and *Microcystis aeruginosa*

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Atmospheric carbon dioxide (CO_2) levels have increased from 280 (ppm) 150 years ago, to 398 ± 3 ppm currently, and may reach 450 (ppm) by 2040 if the rate of the last decade (2 ppm per year) continues. Photoautotrophs are capable of consuming high quantities of CO_2, yet comparative studies of the growth response different CO_2 concentrations elicit in ubiquitous freshwater cyanobacteria genera are lacking. In this study, we cultured two cyanobacteria taxa within controlled CO_2 environments at pre-industrial (180 ppm and 280 ppm) and projected (450 ppm and 1000 ppm) levels. *Anabaena circinalis* and *Microcystis aeruginosa* were the experimental organisms used in the laboratory experiment, and growth rate and biovolume differed as a consequence of the adjusted CO_2 conditions, in particular, increased biovolume was significantly correlated to projected CO_2 levels. The pH values in the upper (1000 ppm CO_2) treatment were on average 2.0 pH units less than the other experimental units, despite yielding high cell counts. We reason, therefore, that projected CO_2 levels at 1000 ppm could affect photosynthetic performance, CO_2 assimilation and base neutralizing capacity. The findings from this study suggest rising atmospheric CO_2 levels will contribute to advanced onset and escalation of cyanobacteria biomass in freshwater ecosystems.

S40.2

Climate, filamentous cyanobacterial dynamics and salinity

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New climate-change scenarios for the Baltic Sea show that water temperature will increase at one of the world's fastest rate and salinity will decrease. The Baltic Sea is also subject to lasting eutrophication with recurrent algal blooms and hypoxia. The phytoplankton biomass is projected to increase with time. At present, salinity constrains the distribution of toxic cyanobacteria northwards but the combined effect of projected temperature and salinity changes on cyanobacterial dynamics remains unclear. Here, we examine the effect of salinity (3, 5, 7) on the N_2-fixing *Nodularia spumigena* specific variation in growth, toxin gene expression, morphotype, peptide profile and competitive interactions. We used a combined approach of short and long-term adaptation to reduced salinity. Large differences in growth and toxin gene expression among genotypes indicated that salinity alone does not explain the low frequency and magnitude of blooms in low salinity waters. Many genotypes exhibited shorter filaments and decreased buoyancy in response to reduced salinity indicating high plasticity. These changes in physical properties suggest that *N. spumigena* could be within the range of available prey for zooplankton. Low salinity favoured the number of heterocysts per filament (e.g. N_2-fixation) and nodularin gene expression did not vary considerably with salinity, suggesting that nitrogen plays a role in the transcription of nodularin. Genotypes with a restricted peptide profile displayed high tolerance to lower salinity and a strong competitive ability against co-occurrent strains. Low salinity favoured *N. spumigena* genotypes that could alter food web efficiency in response to temperature and eutrophication.

S40.3

Limnothrix in Australia

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Limnothrix spp. (Pseudanabaenaceae) are widespread throughout Australia and found in a range of habitats from shallow pools, hot water springs to large reservoirs. These small filamentous cyanobacteria are found in pristine catchments, large rivers and shallow ephemeral streams as well as highly anthropogenically impacted sites. In the last few years, Australian strains genetically consistent with *L. redekei* but also similar to *Geitlerinema* were documented as toxin producing. The first suspicion of toxicity came following cattle deaths in the presence of a unispecific bloom of this material in the Capella area of Queensland. Whilst the toxicology was assumed to be the result of exposure to cylindrospermopsin, no known cylindrospermopsin producer was detected in the sample. A decade later, a strain was isolated from near this location and the mammalian toxicity established.
Limnothrix has been detected in the Fitzroy River Barrage Impoundment where it formed deeper layers in the water column below those of *Cylindrospermopsis raciborskii*. It was well established within the Mackenzie River,- a tributary of the Fitzroy, following major flooding and the late summer pumping of water from a flooded mine pit. Recent field studies have identified *L. redekei* in highly variable conditions including conductivity (50 to 9800 μScm^{-1}), temperature (17 to 35 ^{o}C), pH (6 to 9), turbidity (2 to 170 NTU), ammonium nitrogen (5 to 130 μgL^{-1}), oxidized nitrogen (5 to 60 μgL^{-1}) and sulfate (1 to 1070 mgL^{-1}).
Is this genus the master of variability and climate change, - the new invasive?

S41.1

Use of the Environmental Sample Processor (ESP) and Imaging Flowcytobot (IFCB) in studies of toxic *Alexandrium* blooms in estuaries and coastal waters

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Blooms of the toxic dinoflagellate *Alexandrium fundyense* cause recurrent outbreaks of paralytic shellfish poisoning in the New England region of the eastern U.S. Blooms can be widespread, covering hundreds of km of coastline, or more localized in embayments and estuaries. In recent years, our studies of *Alexandrium* dynamics in both of these systems have incorporated two novel biosensors – the Environmental Sample Processor (ESP) and the Imaging FlowCytobot (IFCB). The ESP uses molecular assays to detect and analyze cells and toxins whereas the IFCB is an automated underwater microscope. Each has presented logistical challenges of different types, requiring new mooring and platform designs, communication protocols, deployment strategies, and assay technologies, to name but a few. This talk will review the manner in which these instruments have augmented our harmful algal bloom (HAB) research in the two hydrographic systems over the last 3-4 years, emphasizing the unique nature of the data each sensor can provide, as well as the engineering and scientific obstacles that had to be overcome or that still remain. Results are highly promising, and suggest that sensors like these can play a complementary role in HAB research and monitoring.

S41.2

Characterizing phytoplankton distributions using targeted sampling by an autonomous underwater vehicle

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Phytoplankton distributions in complex and dynamic coastal environments are highly variable and difficult to characterize and sample. To meet this challenge, we used the Dorado autonomous underwater vehicle (AUV) to (1) create 3D high-resolution maps of the water column that describe variability in phytoplankton distributions and their physical environment, and to (2) autonomously collect water samples within and outside of phytoplankton patches (Fig. 1; Zhang et al. 2010). This technology, with onboard multidisciplinary sensors for physical, chemical and optical measurements, has been applied to phytoplankton ecology research in two HAB hotspots along the California coast: Monterey Bay and San Pedro Bay. Sampling took place during periods of relatively low phytoplankton abundance, providing the opportunity to examine background community composition. A commonly observed pattern was subsurface phytoplankton layers; such layers are hypothesized to harbor seed populations of various HAB species that can be transported to surface waters wherein they rapidly proliferate. Because these bays regularly experience blooms of *Pseudo-nitzschia*, efforts were concentrated on species identification within this genus (via culturing, DNA fingerprinting, SEM), and on domoic acid measurements. Initial assessments indicate higher diversity within the *Pseudo-nitzschia* community in San Pedro Bay compared to Monterey Bay. Further, high species diversity over small spatial scales was observed in San Pedro Bay. Additional information from regional ocean observing systems and shipboard observations provide a contextual picture that describes the environments studied.

S41.3

Follow up to MIDTAL: µaqua, MICROCOKIT and SMS

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In the EU project MIDTAL, a phylochip for toxic algae was developed and field tested in 5 European countries. This universal microarray, now patented, is commercially available. Free Starter kits containing 5 free slides are available and a beta test version is available with 20 slides. The identical protocol was transferred to another project: µaqua to detect freshwater pathogens: bacteria, cyanobacteria, protozoa and diatoms (for assessing water quality). Relevant to HABs is that this phylochip will detect *Planktothrix, Anabaena, Aphanozomenon, Cylindrospermopsis, Microcystis, and Nodularia* as well as their toxins. The toxin genes are on another chip because of the low level of expression (microarray signal) and we have developed a never-before-tested method of retro-transcription directly on the array prior to scanning and this method increased the signal from the expressed toxin genes up to 10 fold. This array will be tested in 6 European countries within this project. This array has been transferred to another European project, MICROCOKIT, where it will be tested over 2 years from 4 sites (pristine, anthropogenic, industrial, and agricultural) along the Tiber River to the Mediterranean Sea. MIDTAL porbes will be transferred to another European project, SMS, where these probes will be used as the capture probes in a sandwich hybridisation assay using electrochemical detection as was developed in the EU project ALGADEC. Novel to this project is the development of a high density microelectrode array (MEA) detecting up to 100 probes with microfludics for final adaptation to a buoy.

S42.1

The critical role of winter chilling in entraining the annual germination rhythm in *Alexandrium fundyense* cysts from a shallow estuarine system

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Blooms of the toxic dinoflagellate *Alexandrium fundyense* occur in coastal waters throughout the world and are prevalent in the northeastern Atlantic, including open ocean and estuarine ecosystems. Blooms in this region are seasonal, due in part to life cycle alternations between motile, vegetative cells and benthic, resting cysts. Newly formed cysts have a mandatory dormancy period during which germination is not possible, but once mature, the resting state will continue if temperatures are unfavorable or oxygen is unavailable. An endogenous annual clock, capable of overriding an otherwise favorable environment for germination, controls seasonal germination of *A. fundyense* cysts in the deep coastal waters of the Gulf of Maine. However, the mechanisms controlling excystment in shallow estuaries are poorly known, and may involve external rather than endogenous factors. Here, we demonstrate that a cold pre-treatment can entrain the endogenous clock of *A. fundyense* cysts collected from the Nauset Marsh System, an estuary on Cape Cod, MA, USA. In a manner analogous to the seeds of temperate plants, a specific amount of “chilling” – defined as exposure to non-freezing low temperatures – is required to confer the ability to germinate. Therefore, the annual germination rhythm contains an endogenous timekeeping component, but requires exogenous cues for sustaining the oscillation and synchronizing it to local seasonal time. This discovery suggests that the timing of the germination clock is determined by winter severity, which helps to explain differences in bloom timing between years, and provides insight into potential responses of cyst-forming harmful algal bloom species to climate change.

S42.2

Alexandrium cyst distribution and germination in Puget Sound

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The Puget Sound *Alexandrium* Harmful Algal Bloom (PS-AHAB - http://www.tiny.cc/psahab) Program investigated cyst dynamics of the toxic dinoflagellate *Alexandrium catenella*. Spatially detailed mapping of cyst abundances in surface sediments during the winters of 2011, 2012, and 2013 identified two shallow (~20 m) "seed bed" areas; Bellingham Bay in the north and Quartermaster Harbor in central Puget Sound. However, the viability of cysts at these seed bed areas was low – with fewer than 54% of cysts germinating when incubated at favorable temperatures. Laboratory experiments designed to investigate both environmental and endogenous controls on cyst germination found excystment to be enhanced by warmer temperatures and light with a hint of a seasonal cycle in excystment that may be due to an endogenous clock. The year-long (2012-2013) experiment found the highest values of excystment to occur in April to October, with minimum values occurring in December/January. Up to 30% of cysts germinated during the minimum period, suggesting that endogenous control on excystment is not tight. A monthly time series of cyst abundances at two locations in Quartermaster Harbor from 2012-2013 found cyst abundances to vary by a factor of ~6 with the lowest cyst abundances occurring in the spring (Apr) bloom season and the highest cyst abundances occurring in late fall (Oct/Nov). These results indicate that cyst abundances are relatively stable over the winter season, but winter cyst abundances *and* viability must be taken into account in order to predict the likelihood of toxic *A. catenella* blooms the following season. The improved understanding of the processes that govern cyst germination and bloom initiation provided by this study contribute towards the development of a predictive capacity for *A. catenella* blooms in Puget Sound.

S42.3

Dinoflagellate cyst assemblages and harmful algal species from different transitional areas of the Western Mediterranean Sea

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The present study identified and quantified dinoflagellate resting cysts in sediment samples from transitional ecosystems in the Western Mediterranean Sea. The studied areas were two estuarine bays along the Catalan coast (Spain), and three lagoons along the western coast of Sardinia (Italy). Samplings were carried out from 2009 to 2011 at 5-11 sampling stations in the different sites. Germination experiments and genetic techniques were used to confirm species identification. Cyst assemblages showed high diversity, especially in the lagoons. However, the Peridiniales group dominated all the assemblages. Numerous species had not been recorded previously in the water column in their respective sites. Moreover, several species were detected for the first time in the Mediterranean Sea, including a new *Scrippsiella* species. Fourteen morphotypes belonged to potentially toxic or noxious species, with the genus *Alexandrium* dominating. Among the harmful species, a new *Alexandrium* cyst morphotype was described. *Kryptoperidinium foliaceum* cysts were first signalled in the Mediterranean Sea. Moreover, several species (e.g. *Vulcanodinium rugosum*, *Gymnodinium litoralis* and *Alexandrium catenella*) were detected for the first time in some of the sites. Detailed studies on cyst assemblages, combined with additional techniques, provided new valuable information on the dinoflagellate communities and, in particular, on harmful species.

S43.1

A different kind of HAB: the world's largest green-tides caused by expansion of coastal seaweed culture in China

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Annually recurrent green-tides in the Yellow Sea since 2007 have been shown to result from direct disposal into the sea of fouling *Ulva prolifera* from *Porphyra* aquaculture rafts on intertidal sand flats along the Jiangsu province coastline. These are the largest macro-algal blooms ever recorded, covering more than 4,000 km^2 and affecting 40,000 km^2 at their peak. The resultant green-tides along the Shandong Province coastline, including the large coastal city of Qingdao have caused significant economic impact. The costs for clean-up and emergency response to the 2008 bloom were estimated at least US$325 million and consequential losses to aquaculture production US$130 million. Significant losses to tourism also occur each year. We tracked the source of the 2008 and 2009 blooms to an area of the Jiangsu Province coastline over 200 km south of Qingdao, where there had been rapid expansion of *Porphyra* aquaculture to as much as 13 km offshore, prior to the appearance of the first bloom in 2007. In excess of 5000 tonnes per annum of waste *Ulva* from the *Porphyra* aquaculture provides sufficient source biomass for the resultant blooms. The waste algae floats, forming patches which expand as they are transported north to Shandong Province where they cause widespread economic impact. Eutrophication of the Yellow Sea is an intractable problem and thus improved aquaculture waste disposal methods in the southern area of Jiangsu Province are likely to reduce or prevent the Yellow Sea green-tides and present a more feasible solution to a recurrent problem.

S43.2

Alexandrium minutum bloom dynamic story along the Brittany coast, environmental control in an invasive framework concept

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The dinoflagellate *Alexandrium minutum* is a bloom-forming species which is distributed worldwide and frequently identified along the French coasts. It is responsible for outbreaks of Paralytic Shellfish Poisoning events that affect aquaculture industry, human health and ecosystems. Contaminations have appeared in Brittany in the late-1980s. Since then, a large time series dataset has been created which combines environmental, phytoplankton and toxicity variables from REPHY French monitoring program and other research programs. The Penzé estuary site is the most documented site. Maximal *A. minutum* abundances recorded in this ecosystem have typical lag-boom-bust periods also reported for invasive animals and plants. The lag period (1988-1992) shows the first apparition of *A. minutum* cells with no toxic events and low densities, the boom phase (1993-2003) exhibits blooms of several millions of cells per liter and regular PSP events, and the bust phase (since 2004) shows irregular and lower densities and no toxic events. Environment control changes over periods from an abiotic control to a biotic control. We will present as well a synthesis of *A. minutum* occurrences in Brittany and different results obtained by statistical method to exhibit the bloom dynamics and the environmental drivers (environmental niche of *A. minutum* populations, *A. minutum* phenology events…).

S43.3

Palaeoecological analysis of phytoplankton regime shifts in response to coastal eutrophication

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We used a multiple-proxy palaeoecological method to reconstruct a 100 yr time series showing coastal eutrophic processes and phytoplankton responses. Total organic carbon, total nitrogen, diatom frustules, dinoflagellate cysts, brassicasterol and dinosterol were extracted from chronologic sediment cores in Sishili Bay, a polluted area in China. The cores showed that eutrophication occurred during about 1975 to 1985, which corresponds to increased human activity associated with China's economic development since 1978. During eutrophication, the biomass of diatoms and dinoflagellates increased, and dominant species shifted abruptly. The small, heavily silicified diatoms *Cyclotella stylorum* and *Paralia sulcata* gradually took the place of the large dominant diatom *Coscinodiscus radiatus*, while dinoflagellates displayed a progressive increase since 1975. Compared to changes in temperature and rainfall during 1950 to 2010, increased fertilizer use, marine aquaculture and sewage discharge showed a better match to the increasing trend in biomass, species shift and nutrient concentration. Altered nutrient supply ratios caused by increased nitrogen inputs play an important role in the shifts in diatom and dinoflagellate assemblages.

S43.4

Victorian whole of government approach in responding to harmful algal blooms in the Gippsland Lakes

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The Gippsland Lakes (the Lakes) are the largest inland water body in Victoria which supports both a commercial and recreational fishery. The Lakes are an important contributor to regional tourism and support the commercial fishery which provides 80-95% of the total Victorian bream catch and 20-50% of the total recreational bream catch. Harmful algal blooms have been recorded within the Gippsland Lakes since the mid 1980s, with the most recent blooms occurring in 2011-2012 and 2012-2013. Harmful algal blooms significantly impact on the Gippsland region with economic losses as a result of the 2011-2012 algal bloom exceding $15 million. In 2011, the Victorian Government began working collaboratively with local industry in response to a prolonged bloom of *Nodularia spumigena*. This model was subsequently refined and adopted in the Victorian government and industry response to the 2012-2013 *Nodularia spumigena* bloom the subsequent year.

S43.5

Decline on long-lasting cyanobacteria dominance in a tropical reservoir related to the reduction of phosphorus and retention time

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High and constant nutrient loads promoted drastic eutrophication process in a tropical reservoir (Funil reservoir, Brazil). A shift in a prior dominance of green algae towards a long standing dominance of potentially toxic cyanobacteria was registered, with annual dominance of three species: the filamentous *C. raciborskii*, *D. circinalis* and the colonial *M. aeruginosa*. Recent changes have occurred in the reservoir catchment area after earlier studies, promoting reduction on phosphorus loads and water column stability in this system. Thus, in this study we test the hypothesis that the effects of these recent modifications would promote reduction on cyanobacteria biovolume and changes of species dominance in Funil reservoir, using a database with pelagic data from a decade of studies (2002-2012). We analyzed the responses of total cyanobacteria biovolume and of the dominant species. Our results corroborated our hypothesis, as we observed a significant reduction of total cyanobacteria biovolume and of the former main bloom-forming species (*M. aeruginosa*), with no significant alteration in the biovolume of the other two cyanobacteria. Our data shows that reduction in residence time, water column stability and phosphorus at limiting concentrations are together capable to significantly reduce cyanobacteria biomass, but also that species-specific responses must be considered in mitigating actions in water bodies with cyanobacteria dominance.

S44.1

From mouse to mass spectrometer

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Since 2001 the monitoring of harmful algal bloom (HAB) toxins in New Zealand shellfish has been completed using liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS) in preference to mouse bioassay. In New Zealand alternatives to mouse bioassay are needed because the shellfish industry relies upon exports of frozen shellfish products, are regularly exposed to HAB toxins and operate within a strict regulatory environment. The development of 'new' methods began in 2001, but refinement is ongoing; today a method able to detect more than 30 toxins within eight minutes is routinely used. Scientists, regulators and shellfish farmers were involved in decisions that ceased all mouse bioassay monitoring of HAB toxins in New Zealand shellfish in 2010. This final step was realised by adoption of HPLC methods for paralytic shellfish toxins (PSTs).
This presentation will discuss the use of LC-MS/MS to test for HAB toxins in shellfish and monitoring data will be used to demonstrate the effectiveness of this approach compared to mouse bioassay. The processes of development, validation, refinement and quality assurance will be illustrated and the latest developments towards application of LC-MS/MS for the monitoring of PSTs will also be revealed.

S44.2

Selective solid-phase extraction of gonyautoxins 2,3 from the dinoflagellate *Alexandrium tamarense* using molecularly imprinted polymers as sorbents

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A new and selective sorbent for molecularly imprinted solid-phase extraction (MISPE) was developed for the isolation of gonyautoxins 2,3 (GTX2,3) from *Alexandrium tamarense* sample, using HPLC coupled to fluorescence detection. The molecularly imprinted polymer microspheres were prepared by suspension polymerization using caffeine as the dummy template molecule, methacrylic acid as the functional monomer, ethylene glycol dimethacrylate as the cross-linker and polyvinyl alcohol as the dispersive reagent. The imprinted polymer microspheres were evaluated further by batch rebinding experiments. The results revealed the polymers exhibited high affinity, selectivity for GTX2,3 and were employed for the solid-phase extraction of GTX2,3. An off-line MISPE method followed by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) for the analysis of gonyautoxins 2,3 was established. Furthermore, the extract samples from *Alexandrium tamarense* were analyzed. The results showed that the interference matrix including C1,2 and GTX1,4 in the extract were obviously cleaned by the MISPE procedure and the extraction efficiency of gonyautoxins 2,3 in the sample ranged from 81.74% to 85.86%.

S44.3

Effect of salinity and pore size on adsorption of diarrhetic shellfish toxins (DST) by HP20 resin

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Okadaic acid (OA) and its dinophysistoxin (DTX) analogues, referred to as diarrhetic shellfish toxins (DST), can be accumulated by molluscs to high levels. Solid phase adsorption toxin tracking (SPATT) technology was developed and adopted in previous studies in order to continuously monitor these toxins in seawater. In this study, OA and DTX1 toxins extracted from the dinoflagellate *Prorocentrum lima* were dissolved in artificial seawater at three different salinities (one-third, half, and natural seawater salinity 27‰) and adsorbed by HP20 resin over a 12-hour time-course experiment. Dynamic adsorption curves showed that toxin adsorption could be described well by a pseudo-secondary dynamic equation. Both toxins were adsorbed most quickly in seawater at half salinity, in which about 97% of the total toxins were adsorbed by HP20 resin within 4 hours. These results demonstrated that adsorption of toxins from seawater onto resin can be affected by salinity. Comparison of pore size distribution of resins tested by a nitrogen sorption system before and after toxin adsorption indicated that toxin molecules were retained by micropores and mesopores below 10 nm in size for the one-third and natural seawater salinities. However, the volume of these pores below 10 nm in half salinity seawater did not change significantly. Resin surfaces observed by scanning electron microscopy demonstrated that more toxin or other matrix aggregates were displayed on the resin surface in half salinity seawater, which was consistent with the relatively constant micropore and mesopore volume of the resin. It is possible that the differences observed in toxin adsorption were related to the solubility of lipophilic toxins at different salinities.

S44.4

Developing Solid Phase Adsorption Toxin Tracking (SPATT) as a monitoring tool for paralytic shellfish poisoning toxins in the Philippines

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Solid Phase Adsorption Toxin Tracking (SPATT) method of monitoring toxic algal blooms has been carried out around the world. In the Philippines, passive solid-phase sampling device has been evaluated as an effective monitoring tool for detecting Paralytic Shellfish Toxins (PSTs). Two polymeric resins (Sepabead SP700 and Diaion HP2MG) were optimized for reliability and sensitivity of the method through series of laboratory and field experiments. Sepabead SP700 had significantly higher PST adsorption capacity than Diaion HP2MG. Among extraction solvents, recovery efficiency of PST was highest in 50% MeOH in both resin types compared to 25% MeOH (pH 3.5) and 3mM HCl. Further acidification (pH 3 – 4) of MeOH before extraction yielded significantly higher PST desorbed in Diaion HP2MG while the opposite was true in Sepabead SP700. Time series adsorption and desorption experiments of the two resin types showed high adsorption and desorption efficiency after 7-day exposure to PST-causing microalgae with 16 hours of solvent extraction, respectively. In the field, SPATT deployed in Bolinao, Pangansinan and Abucay, Bataan during *Alexandrium* spp. blooms effectively detected the presence of PSTs which was highly correlated to *Alexandrium* cell density, plankton and shellfish toxicity. Overall, results supported the potential of SPATT as an effective monitoring tool for PSTs during algal blooms. Further refinements (e.g. time-integrated field sampling) complemented with other monitoring strategies (i.e. plankton monitoring and shellfish toxin analysis) could help in the development of SPATT as a sentinel for toxic algal bloom detection in the Philippines.

S44.5

Algal toxins in seawater: recent developments in detection techniques with a focus on passive sampling to trace pelagic and benthic microalgae

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Algal toxins can accumulate in fish or shellfish and render these foodstuffs unfit for human consumption. Knowledge of the total and dissolved toxin contents helps: (i) understanding the ecological role of toxic algae, (ii) modelling of toxin uptake by aquatic organisms and (iii) estimating the risk of toxins in filtered seawater for land-based aquaculture plants or desalination plants.
Liquid chromatography coupled to high resolution mass spectrometry was used for non-targeted detection of toxins in different passive sampling materials (Oasis HLB, Strata-X, BondElut C18, HP-20; low density polyethylene (LDPE) and silicone rubber (PDMS)). Sorbents were evaluated both in the laboratory (SPE and passive sampling) and in the field (passive sampling), allowing for classification according to accumulation rate and capacity. Deployment of passive samplers at Ingril, a French Mediterranean lagoon, demonstrated that passive sampling is an appropriated technique for screening of toxins from benthic algae since both PnTX-G (from *V. rugosum*) and Dinophysistoxin-1 (from *P. lima*) were quantifiable. The non-targeted detection technique permitted the detection of several hundreds of compounds in algal extracts or on passive samplers, including primary algal metabolites (e.g. lipids) and toxins (e.g. azaspiracids, okadaic acid, palytoxins, pectenotoxins, pinnatoxins (PnTXs) and spirolides). However, most compounds (typically >80-90%) remained unidentified or could only be tentatively identified using natural product libraries.

S45.1

Characterizing toxic activity from *Heterosigma akashiwo*: a tale of two assays

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Blooms of the raphidophyte, *Heterosigma akashiwo* Hada (Sournia) have caused severe economic damage to fish farms in the inland waterways of Washington State, USA, and British Columbia, Canada and are believed to be increasing in frequency and severity. The elements of toxic activity associated with blooms of *H. akashiwo* are not well understood, nor are the environmental factors promoting toxicity. In our study, two laboratory tests were used to characterize *H. akashiwo* toxicity - a modified rainbow trout gill cell (RT-gillW1) assay and embryonic zebrafish exposures. The gill cell assay demonstrated that the *H. akashiwo* toxin is primarily intracellular, highly soluble in methanol and ethyl acetate, and pH stable, with no loss of activity upon storage for months at -20^{o}C. Environmental factors associated with elevated *H. akashiwo* toxicity included nutrient stress and decreased salinity. *H. akashiwo* extracts were used to characterize the toxin's specific cellular targets on the development of zebrafish embryos. As early as 24 h postfertilization (hpf), extracts from stationary phase cultures of *H. akashiwo* caused dose-dependent embryo death and early hatching. Within 48 hpf, intrinsic and specific effects to cardiomyocytes included reduced heart rate and atrial dilation, leading to pericardial edema. Zebrafish heart chambers were formed normally, suggesting that the *H. akashiwo* toxin does not affect early cardiac development but is a physiological poison. In summary, the non-labile toxin from *H. akashiwo* is a largely intracellular, pH stable, polar organic compound that causes impairment of cardiac function in fish.

S45.2

The induction of toxicity in laboratory cultures of *Heterosigma akashiwo* from Puget Sound, WA

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The raphidophycean flagellate, *Heterosigma akashiwo*, (Hada) Sournia is associated with extensive finfish mortality at commercial aquaculture operations in Puget Sound, Washington, and may also affect wild nekton populations in the Salish Sea waters of British Columbia and Washington. The environmental factors thought to influence the toxicity of four Puget Sound isolates of *H. akashiwo* were examined in laboratory batch cultures. As an extension of our work on the effect of environmental factors on the growth of these isolates, we investigated factors that directly influenced toxicity. Toxicity was evaluated using a rainbow trout gill cell line (RT-gillW1). Cells were grown under defined nutrient conditions: varying N and P concentrations, and N:P ratios under two different temperatures that represent the temperature of bloom initiation and bloom maintenance. Compared with other environmental stresses (e.g., salinity, pH, nitrogen sources, and Fe availability) the dominant predictor of cellular toxicity was the interaction between temperature and phosphorus availability relative to the concentration of nitrogen. We also established that the timing of toxicity in the cell growth pattern was generally maximal as cells approached their stationary growth phase, and was short-lived. These results will be discussed relative to conditions expected in Puget Sound where blooms of this ichthyotoxic flagellate are recurrent.

S45.3

Growth promotion of *Heterosigma akashiwo* by marine microorganisms; implication of marine bacterium in bloom formation

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Growth of bloom forming algae is known to be regulated by various factors, such as temperature, salinity, nutrition condition, carbon dioxide concentration, and light intensity. In addition, several studies have demonstrated that marine microorganisms, such as bacteria, play pivotal roles in the regulation of harmful algal bloom. To gain deeper insights into such interspecies interactions, we established six axenized *Heterosigma akashiwo* (*Ha*) strains, and isolated several marine microorganisms from non-axenic *Ha* strains. Among the isolated marine bacterium, there were two strains related to *Winogradskyella poriferorum* and *Spongiibacterium flavum*. When tested on axenized *Ha* strains, *W. poriferorum* showed growth promoting effect on all the *Ha* strains, while *S. flavum* promoted *Ha* growth in strain specific manner. Interestingly, while several reports have demonstrated the existence of algicidal bacterium, very limited number of articles has reported on a bacterium strain that facilitates raphidophyte propagation. Our results demonstrated the existence of more bacterial strains that facilitate growth of bloom forming algae. Interaction of such bacterium with bloom forming algae in strain specific or non-specific manner may be important factor to trigger harmful algal bloom in environment.

S45.4

Ichthyotoxic *Karlodinium* cf. *veneficum* in the Swan-Canning estuarine system (Western Australia): towards management through understanding

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Blooms of the potentially ichthyotoxic dinoflagellate *Karlodinium* cf. *veneficum* have been recorded in the Swan-Canning Estuarine system (Perth, Western Australia) since the late 1990's. These blooms have been associated with large fish-kill events however on other occasions no fish mortalities have been recorded. In 2012 a persistent bloom of *K. veneficum* affected the Swan Estuary for a period of 100 days through the main part of the Austral winter. The bloom started in the upper reaches of the system and moved more than 25 km downstream due to flushing by sporadic rains. Three separate fish-kill events were associated with the bloom. Histological investigation found severe cytotoxic impact to the gill lamellae of impacted fish. The resident *K. veneficum* population has historically produced 6-oxo-KmTx2 karlotoxin however a novel and potentially more toxic karlotoxin cogener was detected during the later stages of the bloom. Periods of moderate to strong water column stratification appear to favour bloom development however there are a number of physicochemical factors that control growth of *K.* cf. *veneficum*. Understanding bloom dynamics and toxin profile are important for developing and applying effective control techniques.

S45.5

Oxygen uptake of European plaice (*Pleuronectes platessa*) is reduced during exposure to the toxic haptophyte *Prymnesium parvum*

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During the last century, blooms of the toxic haptophyte *Prymnesium parvum* have been responsible for massive fish kills in both aquaculture and wild populations. Despite decades of research, the ichthyotoxic properties of *P. parvum* and how this species exactly kills fish are still debated in the literature. We developed an instrument (DeMeVox) that reduces the effects of handling and stress of flatfish, during measurements of oxygen extraction and ventilatory flow. Fourteen specimens of European plaice (261 ± 40 g) (*Pleuronectes platessa*) were exposed to progressive hypoxia. After a recovery period, seven fish were exposed to lethal concentrations of *P. parvum* (154×10^3 - 320×10^3 cells ml^{-1}) and seven fish to the non-toxic control alga *Rhodomonas salina* (249×10^3 - 468×10^3 cells ml^{-1}). During exposure to progressive hypoxia, the fish were able to compensate for the lack of dissolved oxygen by increasing their ventilatory flow. However, this was not possible during exposure to bloom cell concentrations of *P. parvum*. Results from the fish exposed to *P. parvum* showed a substantial decrease in oxygen extraction (56.4 – 46.1 % of ambient pO_2) within a few hours, whereas the fish exposed to *R. salina* in similar cell concentrations were unaffected. These results strongly indicate that the European plaice dies from suffocation when exposed to lethal concentrations of *P. parvum*, even though the surrounding water is fully oxygenated. A combination of gill damage and buildup of mucus on the gills during the exposure may explain the decreased oxygen uptake.

S46.1

HAB toxins: past, present and future concerns for UK aquaculture

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The Centre for Environment Fisheries and Aquaculture Science (Cefas) is one of the partner organisations contracted by the Food Standards Agency (FSA) in the United Kingdom (UK) for the analysis of harmful toxins accumulating in bivalve shellfish. For many years Cefas has conducted routine monitoring of shellfish using a variety of methodologies, with the primary focus on the determination of a range of regulated lipophilic toxins together with the toxins responsible for Amnesic Shellfish Poisoning (ASP) and Paralytic Shellfish Poisoning (PSP). With a move away from reliance on biological assays for shellfish testing, use of analytical instrumentation has provided opportunities to collate valuable data on the occurrence and distribution of specific toxin groups and their associated profiles around the UK. This presentation will focus on the HAB toxins of concern within UK waters based on data generated over recent years. Information will be presented illustrating the high variability in temporal and spatial occurrence of toxic events, together with associated toxin profiles. The question regarding the potential increase in occurrence of HAB events will also be discussed. Finally, the importance of appropriate sampling and monitoring regimes will also be highlighted through the lessons learned following recent intoxication episodes.

S46.2

Azaspiracid detections in New Zealand shellfish

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The first recorded human azaspiracid (AZA) poisonings occurred in 1995, following the ingestion of contaminated shellfish from Ireland. AZA compounds have since been detected mostly in shellfish from Northern Europe. It is now known that AZAs are produced by dinoflagellates of the genus *Azadinium*. In New Zealand, the shellfish toxin monitoring programme for lipophilic toxins is performed using LC-MS/MS analysis. It has included the regulated compounds AZA-1, AZA-2 and AZA-3 since 2002. Although there have been no reported AZA poisonings to date, the first reportable detection was of AZA-2 in dredge oysters (*Tiostrea chilensis*) harvested from the NZ south-east coast in 2011. More infrequent low-level detections have since been recorded, in numerous shellfish species from different areas. Efforts made to identify the causative micro-algae have so far been unsuccessful. Isolations of *Azadinium*-like cells have proved to be *Heterocapsa niei*, which is genetically closely related to the *Azadinium* genus and has a very similar appearance. This talk will present a summary of events to date and illustrate the efforts made to mitigate the risks posed by these toxins and ensure the ongoing safety of New Zealand shellfish to global consumers.

S46.3

Recent advancements in measuring exposure to algal toxins

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One of the primary missions of the Division of Laboratory Sciences (DLS) is to provide laboratory support that improves the rapid and accurate detection and diagnosis of human exposure to chemical agents and selected natural toxins. DLS evaluates biomedical samples for exposure to select toxins in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and continues to work in partnership with both the Centers for Medicare and Medicaid Services and the U.S. Food and Drug Administration in support of clinical laboratory quality. DLS also provides support and administration for the Chemical Laboratory Response Network (LRN-C) including reference materials testing, partner laboratory training, and proficiency testing maintenance for distributed methods. Current algal toxin methods supported by DLS include analysis of human urine for saxitoxin which is listed as a select toxin under Health and Human Services Code of Federal Regulations. The DLS saxitoxin method was used to confirm cases of paralytic shellfish poisoning in Alaska during 2011. Since the saxitoxin method can rapidly identify paralytic shellfish poisoning, its technology continues to be transferred to a select number of partner laboratories in order to facilitate the work of public health authorities and complement the many shellfish monitoring programs worldwide.

S46.4

Harmful algal bloom events in Malaysia, 2013-2014

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In 2013-2014 harmful algal blooms occurred in several areas of Malaysian waters in a big way after several years of absence. From September 2013 until February 2014, blooms of the toxic dinoflagellate *Pyrodinium bahamense* persisted on the west coast of Sabah causing several cases of human toxicity. In 2013 the first ever event of *P. bahamense* bloom also occurred on the east coast of Peninsula Malaysia in the waters of Kuantan, Pahang. The event also caused human toxicity. Serious economic losses were caused by a series of massive cage cultured fish kills caused by dinoflagellate blooms in both Sabah and Peninsula Malaysia. In Sabah the causative species were *Noctiluca scintillans* and *Cochlodinium polykrikoides*. The *Noctiluca* bloom was interesting because it was the first time that the bloom was of the red/orange variety rather than the normal green. In the Johore Straits, the fish kills were caused by *Karlodinium* spp. In March and April of 2014 there were also massive blooms of *Gonyaulax spinifera* on the west coast of Sabah but there was no evidence of adverse impact. The increase in frequency and locations of these harmful algal blooms pose a serious challenge to the expansion of shellfish and finfish aquaculture in Malaysia.

S46.5

Paralytic shellfish monitoring in the Philippines - a management review

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Paralytic shellfish poison has been a perennial problem in the Philippines with significant impacts in public health and the shellfish industry. To mitigate its impact, the Philippine government implements a monitoring program. The core of the monitoring program is the detection of causative organism in the water and analysis of toxin in the shellfish. Capacity building for regional and local testing has been done however; monitoring activities has been restricted by limitations imposed by mouse bioassay. Alternative methods that may be suitable for laboratories in remote sites were evaluated. These methods include ELISA and rapid test kits. Use of these methods is currently incorporated in the regular monitoring procedure in various regional and local laboratories. Current monitoring focuses on the toxin level in shellfish, and advisories and bulletins are released based on the established regulatory limit. However, recent observations suggest that other factors need to be considered such as varied toxin retention time in different shellfish species. Review of monitoring data showed extreme differences in toxin level between different kinds of shellfish. Benthic shellfish species like *Soletellina* sp. continue to retain high toxin level as compared to *Perna viridis*, the indicator species, which are negative for PSP. Results of the comparative study will be used to revise the current PSP monitoring program.

S47.1

The toxicity and phylogenetics of *Ostreopsis* species in temperate New South Wales, Australia

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The genus *Ostreopsis* Schmidt comprise of nine species originally described from the surfaces of macroalgae, seagrass, coral rubble, sand as well as mangroves in tropical regions. In the past decade, *Ostreopsis* species have caused dense blooms in temperate waters and toxic strains have been identified from New Zealand, Japan and the Mediterranean waters. *Ostreopsis* species produce palytoxin (PTX) analogues that are involved in causing human illnesses such as clupeotoxism through the transfer and accumulation in fish, and respiratory problems through aerosol exposure during blooms. Decline in the populations of sea urchins and shellfish have also been reported during *Ostreopsis* blooms, suggesting wide-ranging ecological impacts. Here, we report the identification and culturing of *Ostreopsis* species from samples around the coast of New South Wales, Australia. Microscopy and phylogenetic analysis based on internal transcribed regions (ITS1 and ITS2) along with 5.8S, D1-D3 and D8-D10 large subunit (LSU) rDNA regions were used for identification. The toxin profiles were analysed via LC-MS/MS, and some strains of *Ostreopsis* were found to produce putative analogues of PTX. The implications of these findings for aquaculture industries and for our understanding of the biogeography of *Ostreopsis* are discussed.

S47.2

Inorganic nutrients uptake and organic phosphorus utilization by *Ostreopsis* cf. *ovata*

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Blooms of *Ostreopsis* cf. *ovata* in the Mediterranean Sea are characterized by different intensities and toxicity levels. Dinoflagellates toxin production often reflects the nutrient status of the cells and, under P starvation, the most frequent response consists in increased toxicity levels. Studies on cultured Adriatic strains of *O.* cf. *ovata* showed that cells are less toxic under both N and P depletion, highlighting the importance to better characterize growth dynamics, nutrient uptake and starvation responses. Some other observed aspects are that N and P are rapidly taken up by cells during the exponential phase: P is consumed more rapidly than N during the first days, determining an increase of the N/P ratio in the external medium. At the same time, the cells are able to maintain a quite constant intracellular N/P ratio, with levels not higher than 25, leading to the hypothesis that *O.* cf. *ovata* can obtain nutrients from organic matter. The present study was performed in order to get insights into nutrient utilization by *O.* cf. *ovata*. The characterization of the nutrient uptake kinetics showed that this species has higher Vmax and higher affinity values for P uptake than for N. In addition, a study performed with cultures grown at different N/P ratios, showed that alkaline phosphatase activity is enhanced as soon as external P is depleted. These findings confirm the high efficiency of *O.* cf. *ovata* in nutrient utilization and in P acquisition, aspects that could confer advantages over competing species.

S47.3

Seasonal and multiannual trends of *Ostreopsis* cf. *ovata* in the Gulf of Naples (Mediterranean Sea)

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The expansion and the intensification of *Ostreopsis* blooms are posing new threats to human and environmental health in temperate waters, and particularly in the Mediterranean Sea. Nonetheless, the reasons for this increase and predictions of future trends are still out of our reach. The phenology and ecology of *Ostreopsis* cf. *ovata* were investigated in the Gulf of Naples (Tyrrhenian Sea, Mediterranean Sea) to assess distribution patterns and evaluate factors affecting the development of the blooms. Sampling was carried out weekly from 2007 to date during summer/early autumn, at two stations close to Naples city. Additional samplings were conducted since 2011 at the natural CO_2 vents of Castello Aragonese (Ischia Island) at station with different hydrodynamic conditions (exposed and sheltered). At Naples stations, *O.* cf. *ovata* distribution showed a decreasing trend over the years and a marked seasonality, with a bimodal distribution: a first, more intense bloom in July ($< 3.5 \cdot 10^5$ cells g^{-1} fw) was followed by a second, minor and less constant bloom in late September ($< 2.4 \cdot 10^5$ cells g^{-1} fw). At Castello Aragonese, maximum abundances were recorded in July 2011 at exposed stations ($< 1.1 \cdot 10^6$ cells g^{-1} fw) with significant differences from sheltered ones. Low pH (< 6.8) at some of the stations did not affect the distribution pattern. Temperature, salinity and nutrient availability did not appear to represent primary drivers for bloom timing and intensity, while the exposure likely affects the dynamics of *O.* cf. *ovata* blooms.

S47.4

Changes in marine vegetation and potential facilitation of *Ostreopsis* blooms in the Mediterranean Sea

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The *Ostreopsis* blooms represent an emerging phenomenon in the Mediterranean Sea, catching the attention of the scientific community. Despite the increasing amount of literature on the subject, the driving factors are still poorly known, also due to contrasting results on traditional environmental variables at different spatial and temporal scales. In the framework of a European project (M3HABs: Risk Monitoring, Modelling and Mitigation of Benthic Harmful Algal Blooms along Mediterranean coasts) we are investigating the potential role of human impacts in the facilitation of *Ostreopsis* blooms in coastal areas. Human impacts have direct and indirect effects on marine vegetation that can cause radical changes in structure and composition of macroalgal communities. In the Mediterranean Sea, in recent decades, *Cystoseira* forests have suffered widespread and apparently irreversible loss (regime shifts), much of which may have gone unnoticed. They are usually replaced by *Dictyota* less complex communities, articulated Corallinales turfs or encrusting corallinales deserts (barren grounds), in function of the human driver causing the regime shift. In this talk we review the potential effects of regime shifts in marine vegetation on *Ostreopsis* blooms and test the hypothesis that the complexity of macroalgal assemblages affects the risk of potentially harmful events in the Mediterranean Sea. Some experiments have been set up in the framework of the M3HABs: the experimental protocol and the preliminary results will be discussed.

S47.5

Light-responsive growth of the harmful dinoflagellates

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To understand the bloom dynamics of harmful algae, it is important to clarify the light intensity (= photon flux density) affecting algal growth. General light sources, fluorescent tubes and halogen lamps, have previously been used for research on the light-responsive growth of harmful algae; however, their illumination is incapable of simulating closely both the spectrum and intensity of natural photosynthesis available radiation (PAR) in oceanic water. In order to evaluate bloom dynamics of the harmful dinoflagellates in coastal waters, this study used a newly developed photoirradiation-culture system to closely simulate natural PAR in oceanic water at varying levels of light intensity. We precisely estimated the growth responses of *Heterocapsa circularisquama*, *Karenia mikimotoi* and *K. papilionacea* to various light intensities. These algae grew at the wide ranges of 10^0~10^3 μmol photons m^{-2} s^{-1}. The relationship between light intensities and observed growth rates in single species was calculated at $R > 0.90$ ($P < 0.01$) using a regression analysis with an equation model. The equation determined the critical light intensities, the thresholds and optimum ranges of light intensity, for growth of the algae.

S48.1

Extensive survey on the distribution of the overwintering population of *Karenia mikimotoi* using LAMP method

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In the summer of 2012, a large-scale red tide of the harmful dinoflagellate *Karenia mikimotoi* occurred in the Bungo-Channel, western Japan, and caused a great loss to aquaculture industries. Motile cells of *K. mikimotoi* have seldom appeared in winter in this area, and the resting cyst has not been reported in its life cycle. Where does the seed population of the red tide come from? To elucidate this issue, we conducted an extensive survey to examine the presence of overwintering cells of *K. mikimotoi* using LAMP (Loop-mediated Isothermal Amplification) method. Surface water samples (0-10 m column) were collected 8-16 times at 139 sampling stations along the coast of Ehime and Oita Prefectures from January to March, 2013, and crude DNA of the plankton fractions (>3.0 μm) was extracted by boiling with TE buffer. First screening by LAMP method was conducted by fluorescent detection using a primer set reported by Zhang et al. (2009). To confirm the LAMP specificity, the samples showing positive results in the first screening were checked by annealing curve analysis with the above primer set and a newly designed primer set (Sakamoto, unpublished). At the first screening, many samples had shown to be positive reactivity, and some of them were confirmed to be specific to *K. mikimotoi* by the annealing curve analysis. As a result, overwintering cells of *K. mikimotoi* were detected within a limited area of the coast of Ehime Prefecture, and they may act as a seed population of the red tide in the Bungo-Channel.

S48.2

Assessing the abundance of the ichthyotoxic *Karenia mikimotoi* by quantitative PCR and microscopy

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The dinoflagellate *Karenia mikimotoi* may form blooms causing fish kills in coastal waters of the North East Atlantic region, including Norway and Sweden. A *Karenia mikimotoi* specific quantitative PCR (qPCR) assay was developed, based on the use of SYBR Green 1. Samples were collected monthly from August 2011 to June 2012 in outer Oslofjorden, and during a cruise in April/May 2012 samples were collected at 10 stations in Skagerrak. In the Skagerrak samples the abundance of *Karenia* was either very low or below the detection limit and no observations were made by microscopy in any of the corresponding cell counts or net hauls. *Karenia* was detected by qPCR in all samples from OF2, with a peak in August and September and with very low signals in February, March, April and May. *Karenia* was identified by light microscopy in net hauls from August and September. By cell counts it was only recorded in September. The qPCR assay was more sensitive than traditional counting methods using light microscopy in the detection of *Karenia*. In addition to traditional microscopy based methods, qPCR assays can be a powerful tool in the identification and quantification of *Karenia* and other harmful algae and may prove especially useful if applied in early warning programs.

S48.3

Understanding the fish-killing mechanism of *Alexandrium catenella* in Chilean fjords

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Outbreaks of the fish-killing dinoflagellate *Alexandrium catenella* have caused significant economic losses (~$10M in 2009) to the salmon industry in the south of Chile. However the precise ichthyotoxic mechanism by this PST dinoflagellate remains poorly understood. The rainbow trout cell line RTgill-W1 assay was used to investigate the ichthyotoxicity of multiple *A. catenella* strains under different environmental conditions, and toxicity of whole cells, lysed cells and culture medium compared. Ichthytoxic potency from *A. catenella* was highly variable among strains and strongly correlated to cell-abundance. Lysed cells showed higher toxicity than intact cells and supernatant, reducing gill cell viability down to 20% of controls at 4000 cells ml^{-1}. However, gill cells exposed to pure PST fractions (C1&C2, STX, GTX 1&4) exhibited only limited loss of gill cell viability (<30%), even at equivalent cell concentrations exceeding those detected in southern fjords. The *A. catenella* lytic compounds were pH and temperature stable but rapidly (1-2 days) degraded in the light. Superoxide production was <5 pmol $cell^{-1}$ hr^{-1}, and hence also considered to play an insignificant role. The production of yet uncharacterized lytic toxins and a possible role of PUFA are pursued.

S48.4

First confirmed report of fish-killing *Pseudochattonella* species (Dictyochophyceae) on the west coast of Canada

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In late September 2013 a bloom of *Pseudochattonella* species caused mortality in farmed Atlantic salmon in an aquaculture facility in Quatsino Sound, British Columbia. The bloom was preceded by a storm event with high winds and heavy rain. *Pseudochattonella* cells detected early in the bloom were small round flagellated swimming cells, which developed into larger oval and oblong cells within a few hours. Mortality was observed at this time, and although the bloom persisted for several days it appears ichthyotoxicity was only seen during this period. Extreme pathology of the gill tissue was observed in mortalities, with gills appearing ragged, frayed and mucusy. A culture was established from live samples obtained from the bloom. Quantitative real-time PCR analysis was performed using a published Taqman assay, and the identification of *Pseudochattonella* species in the isolated culture was confirmed. Although *Pseudochattonella* has been implicated in mortalities of farmed salmon in this area since 2007, identification to present has been based solely on cell morphology; this is the first genetic confirmation of that identification.

S48.5

Variable contribution of toxins and superoxide to ichthyotoxicity by harmful marine phytoplankton: an *in vitro* approach

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Selected harmful marine microalgae (*Amphidinium, Chattonella, Heterosigma, Karenia, Karlodinium*) produce largely uncharacterised chemical compounds that affect fish, but the role that reactive oxygen species play in toxicity remains inconclusive. In this study, an *in vitro* assay using fish gill cells was used to quantify the effect of these microalgae on gill cell viability and their antioxidant defences, while simultaneously assessing superoxide production. Known purified algal toxins and crude extracts were also tested. *Chattonella marina* was the most ichthyotoxic species (gill cell viability down to 35%) and the biggest producer of superoxide (14 pmol cell^{-1} hr^{-1}). Gill cells challenged with *Chattonella* also registered a significant increase in activity of superoxide dismutase (maximum of 23%) and lactate dehydrogenase (51.5% TCC), while other species showed lower effects on superoxide dismutase activity (<10%). Enzymatically produced superoxide (levels equivalent to *Chattonella*) could account for 14% of ichthyotoxicity. *Heterosigma akashiwo*, *Karlodinium veneficum* and *Karenia brevis* also affected gill cell viability but concomitant with low superoxide production. Karlotoxin KmTx-2 (0.021-210 ng mL^{-1}) was highly correlated with toxicity by *K. veneficum* (10-10^5 cells mL^{-1}); both showed low toxicity after 2 hrs (32%) but which increased significantly after 5 hrs (≥50%). Brevetoxin PbTx-2 proved to be more toxic than PbTx-3 (LC_{50} of 22.1 versus 35.2 µg mL^{-1}). Pure amphidinol from *Amphidinium carterae* was highly ichthyotoxic, LC_{50}=1.9 µg mL^{-1}. An accounting exercise of the impact of known phycotoxins and superoxide indicates a highly variable contribution to ichthyotoxicity by other compounds (i.e. free fatty acids) including lipid peroxidation products.

Poster Topics

In numerical order

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Toxicology	P-36 – P-46
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Transcriptomics	P-83 – P-86
Gambierdiscus	P-87 – P-90
Marine and Freshwater HABs	P-91 – P-101
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HAB life cycles and physiology	P-104 – P-114
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P-1

Discovery of enzymes related to marine polyether biosynthesis in dinoflagellate extracts

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Ladder-frame polyethers have been assumed to be biosynthesized via polyene and polyepoxide precursors. However, the fused ether ring formation of marine ladder-frame polyethers has not yet been elucidated because of a lack of genetic information of dinoflagellates and the complexity of these structures.
Brevisamide, which consists of a simple 6-membered ether ring, a dienal side chain and an acetylated amine, is produced by the toxic dinoflagellate, *Karenia brevis*. In order to search for an enzyme catalysing ether ring formation several proposed biosynthetic precursors of brevisamide, which have a linear olefin or an epoxide structure, were synthesized and incubated with extracts from the dinoflagellates, *Protoceratium reticulatum* and *Karenia* spp. Although desired 6-membered ether ring products were not detected in the extracts, some oxidation reaction products of terminal side chains were observed when the precursors were incubated with *Karenia* spp. extracts.
A truncated epoxide precursor of yessotoxin was also designed and synthesized from D-glucose. Incubation of the model epoxide with extracts from *P. reticulatum* cells gave rise to a 6-*endo* epoxide-opening product. This result strongly suggested that the 6-*endo* epoxide-opening cyclization was catalysed by an enzyme in the extract from *P. reticulatum*.

P-2

Impact of nitrogen sources on gene expression and toxin production in the diazotroph *Cylindrospermopsis raciborskii* CS-505 and non-diazotroph *Raphidiopsis brookii* D9

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Different environmental nitrogen sources play selective roles in the development of cyanobacterial blooms and noxious effects are often exacerbated when toxic cyanobacteria are dominant. *Cylindrospermopsis raciborskii* CS-505 (heterocystous, nitrogen fixing) and *Raphidiopsis brookii* D9 (non-N_2 fixing) produce the nitrogenous toxins cylindrospermopsin (CYN) and paralytic shellfish toxins (PSTs), respectively. These toxin groups are biosynthesized constitutively by two independent putative gene clusters, whose flanking genes are target for nitrogen (N) regulation. So far it is not known how or if toxin biosynthetic genes are regulated, particularly by N-source dependency. Here we show that binding boxes for NtcA, the master regulator of N metabolism are located within both gene clusters, as potential regulator of toxin biosynthesis. Quantification of intra- and extracellular toxin content in cultures at early stages of growth under nitrate, ammonium, urea and N-free medium showed that N-sources influence neither CYN nor PSTs production. However, CYN and PSTs profiles were altered under N-free medium resulting in a decrease in the predicted precursor toxins (doCYN and STX, respectively). Reduced STX amounts were also observed under growth in ammonium. Quantification of toxin biosynthesis and transport gene transcripts revealed a constitutive transcription under all tested N-sources. Our data support the hypothesis that PSTs and CYN are constitutive metabolites whose biosynthesis is correlated to cyanobacterial growth rather than directly to specific environmental conditions. Overall, the constant biosynthesis of toxins and expression of the putative toxin-biosynthesis genes supports the usage of qPCR probes in water quality monitoring of toxic cyanobacteria.

P-3

Insertions within the saxitoxin biosynthetic gene cluster give rise to differential toxin profiles in the cyanobacterium *Scytonema crispum*

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The saxitoxins are a large group of neurotoxic alkaloids that block voltage-gated sodium channels and are biosynthesised by select species of cyanobacteria and dinoflagellates. The cyanobacterium *Scytonema crispum* was recently identified as a saxitoxin-producer in New Zealand. Saxitoxin profiles vary among strains with *S. crispum* UCFS10 solely producing saxitoxin, while *S. crispum* UCFS15 produces a range of hydroxylated and mono-sulfated saxitoxin analogues. We performed whole-genome sequencing of these two *S. crispum* strains to determine whether their distinct toxin profiles could be explained by differences within the saxitoxin (*sxt*) biosynthetic gene cluster. The genomes of both strains were comparable in size (8 Mb), GC content (42.5%) and the prevalence of transposases and repeat regions. Each *sxt* cluster spanned approximately 50 kb, representing the largest *sxt* gene cluster to date. Additionally, a previously undescribed *sxt* gene, *sxtO2*, was identified. This is an adenysulfate kinase and thought to replace the function of *sxtO.* Alignment of both clusters revealed multiple insertion sequences in *S. crispum* UCFS10's *sxt* gene cluster. Three of these insertions were localised within ORFs, resulting in the truncation of *sxtO2*, *sxtDIOX* (hydroxylation prior to O-sulfotransfer) and *sxtN* (N-sulfotransfer), all thought to be responsible for sulfated saxitoxin analogues. Furthermore, the putative role of *sxtX* in derivation of neosaxitoxin is highlighted as both strains contained the gene but only *S. crispum* UCFS15 produced this variant. This study represents the first insight into a saxitoxin-producing species with differing toxin profiles and may lead to a greater understanding of the proposed saxitoxin biosynthesis pathway.

P-4 ST-08

Detection of cyanotoxin genes in freshwater benthic cyanobacteria from subtropical southeastern Australia using multiplex real time PCR

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Cyanotoxins can represent a risk to human, animal, and ecosystem health when present in large quantities in either drinking or recreational waters. Cyanotoxin production by planktonic cyanobacteria has been well-studied and documented, resulting in an extensive literature. In recent years, more emphasis has been placed on the characterisation of freshwater benthic cyanobacteria, particularly species that form dense proliferations. In 2012 a pilot study of benthic cyanobacterial mats from lakes and streams in subtropical southeastern Australia, up to 25% of samples were found to contain cyanotoxin genes when screened by multiplex real time PCR. An expanded study from comparable habitats has recently shown a relatively high occurrence of the *sxtI* gene, indicating production of paralytic shellfish toxins, and *ndaF*/Anabaena-type *mcyE* gene homologues, indicating production of nodularin and microcystins, respectively. Examination by light microscopy to determine the dominant putative cyanobacteria present revealed the sample positive for the *sxt I* gene was dominated by the genera *Scytonema* and *Lyngbya*, both previously reported to produce saxitoxin, while three samples positive for *ndaF*/Anabaena-type *mcyE* gene homologues were dominated by mixtures of *Anabaena inaequalis, Nodularia moravica, Nostochopsis lobatus, Phormidium tergestinum, Phormidium* sp. and *Oscillatoria sancta,* some of which are known to produce nodularin and microcystin. This study further supports observations from other continents on the relatively widespread occurrence of cryptic cyanotoxicity in the freshwater benthic environment. Candidate toxin-producing genera identified in this study require further characterisation using molecular and morphological techniques to better understand their identity and mode of potential toxicity.

P-5

Development of a sensitive and selective HILIC-UPLC-MS/MS method for high throughput analysis of paralytic shellfish toxins using graphitic carbon SPE

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Routine regulatory monitoring of paralytic shellfish toxins in bivalve shellfish can prove difficult using instrumental techniques. Typical analysis employs oxidative derivitisation and complex liquid chromatography fluorescence detection methods (LC-FL). The pre-column oxidation LC-FL method (AOAC 2005.06) is currently implemented in New Zealand and the United Kingdom. Positive samples require fractionation of the various saxitoxin groups and two different oxidations are required in order to confirm the identity and quantity of each congener present. The method has a low sample throughput, and can be cumbersome and expensive for full quantitation results.

There is a need for alternative methods that provide faster turnaround times and have improved detection limits. Hydrophilic interaction liquid chromatography (HILIC) HPLC-MS/MS has been used for research purposes, however, high detection limits and substantial sample matrix issues have prevented it from being a viable alternative for routine monitoring purposes. We have developed a HILIC UPLC-MS/MS method for paralytic shellfish toxins with an optimised desalting clean-up procedure on inexpensive carbon solid phase extraction (SPE) cartridges for reduction of matrix interferences. This allows sensitive, selective and rapid analysis of shellfish samples for reduced routine monitoring costs, and faster turnaround times. Additionally, this approach avoids the need for complex calculations to determine sample toxicity as each congener is able to be quantified as a single resolved peak.

The novel method has improved limits of detection and quantitation over the existing LC-FL monitoring method. An initial comparison between HILIC UPLC-MS/MS and LC-FL methods show comparable results on naturally contaminated shellfish samples (n=94, R^2=0.97).

P-6

Ultra-performance liquid chromatography/post-column oxidation/fluorescent detection for determination of paralytic shellfish toxins in shellfish

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Paralytic shellfish toxins are a group of potent neurotoxins produced by toxigenic dinoflagellates such as *Alexandrium* and *Gymnodinium* spp. The HPLC/post-column oxidation/fluorescent detection, developed by Oshima (1995), has been widely used as a chemical detection method for measuring the toxins in shellfish and dinoflagellates. One of the drawbacks of the method is that the toxins are quantified by three different chromatographic runs and it takes more time than the mouse bioassay which is the official testing method in Japan. In our present study, we developed ultra-performance liquid chromatography/post-column oxidation/ fluorescent detection (UPLC/OX/FD) for practical use by improving the performance of its operation. The reaction system was improved by using a small mixer and post-reaction coil to improve the efficiency of the post-column oxidation procedure. We also investigated the use of several analytical columns to prove that these are suitable for UPLC/OX/FD. We were successful in improving the performance of the UPLC/OX/FD method and shortening the chromatographic run time.

P-7

Facilitating implementation of the receptor binding assay for PSP

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The receptor binding assay (rba) for paralytic shellfish poisoning (PSP) offers excellent sensitivity, high throughput, and a reliable measure of toxicity. It is far better than the mouse bioassay for PSP and, as a measure of toxicity, simpler and more cost-effective than HPLC. Where HPLC determines the concentration of individual toxins and requires a standard for each, the rba determines net toxicity and requires only a single standard.

- Several steps have been taken recently to facilitate the implementation of the rba:
- The rba is now an AOAC International validated method, OMA 2011.27.
- The efficiency of the method relies on a multiwell plate scintillation counter.
- The radiolabeled saxitoxin required for the rba is now available from American Radiolabeled Chemicals (ARC) in St. Louis, Missouri, U.S.A.

In the U. S., the use of radioisotopes generally requires licensing by the Nuclear Regulatory Commission (NRC). ARC, with assistance from People for the Ethical Treatment of Animals (PETA), has obtained an exemption from the NRC so that labs wishing to use the rba can now purchase, use, and dispose of the radiolabeled saxitoxin without an NRC license. A lab can now simply purchase the radiolabeled saxitoxin from ARC; the procedure differs little from the way one would purchase an ordinary non-radioactive reagent. The membrane suspension needed for the rba is now available from Sigma, frozen in vials, ready for use in the assay. The unit of sale is a box of 60 vials, sufficient for the number of plates that can be run with a single vial of radiolabeled saxitoxin.

P-8

Mass spectrometric screening of novel gymnodimine-like compounds in isolates of *Alexandrium ostenfeldii*

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Gymnodimines (GYMs) are toxic compounds produced by the marine dinoflagellates *Karenia selliformis* and *Alexandrium ostenfeldii*. Though accumulation of gymnodimines in mussels may cause food poisonings, knowledge on the diversity and distribution of gymnodimines in these taxa is still scarce. The only gymnodimine analogue so far reported from *A. ostenfeldii* is 12-methylgymnodimine. We have studied gymnodimine toxins in 22 strains of *A. ostenfeldii* isolated from different geographic locations and belonging to different phylogenetic clades.

Cultures were screened using the Hypersil BDS C8 column chromatographic separation and linear ion trap tandem mass spectrometry with positive mode electrospray ionization. Two separate gymnodimine analogues were found at *m/z* 508 in *A. ostenfeldii* isolates from the Baltic Sea and Saanich (Canada), and only that in the Saanich AOPC strain had retention time and mass fragmentation similar to the gymnodimine reference standard isolated from *K. selliformis*. Three separate analogues at *m/z* 524 were furthermore found in the Baltic Sea and US East coast strains, suggesting that they contain gymnodimine B/C related analogues, which have so far only been detected in *K. selliformis*.

Moreover, three related intensive and some smaller analogue signal peaks were observed with similar retention in MS full scan screening, e.g. 12-methylgymnodimine-like analogue at *m/z* 522 in the US East coast strains only. Almost all investigated isolates produced at least one strong lipophilic compound. The indicated diversity of GYMs emphasizes that a number of different reference chemicals will be needed to comprehensively identify and monitor these toxins in marine samples.

P-9 ST-20

Brevetoxin uptake in co-occurring marine microbes

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We report the uptake of brevetoxin (PbTx), a neurotoxic shellfish poison produced by the marine photosynthetic dinoflagellate *Karenia brevis* (1, 2), in several co-occurring marine microbes. Using confocal microscopy, the brevetoxin congener PbTx-2 conjugated to the fluorescent probe BODIPY (3) is shown to accumulate significantly in lipid-rich regions of certain algal taxa (diatoms and haptophytes), whereas other algal groups (dinoflagellates, cryptophytes, and cyanobacteria) do not significantly accumulate PbTx-2 over a 4-hour time course. This suggests that differences in membrane physiology among various phytoplankton groups influence the degree of PbTx uptake. Zooplankton such as ciliates and rotifers are also shown to exhibit significant PbTx uptake, yet in the former microbial heterotroph toxin localization is more distinct from lipid-rich regions. These data demonstrate a potential for transfer of waterborne PbTx to specific microbial groups and trophic levels during *K. brevis* blooms. Using incubations with unlabeled PbTx-2 combined with LC-MS analysis, we are now testing the hypothesis that intracellular accumulation of PbTx-2 in co-occurring microbial taxa can significantly contribute to losses of waterborne PbTx that have been previously reported (4, 5). Ultimately these findings suggest that PbTx, and potentially other harmful algal metabolites, can be passively vectored through the marine microbial community.

P-10

Lipophilic shellfish toxins in *Dinophysis caudata* picked cells and in shellfish from the East China Sea

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Pectenotoxin (PTX)-group and okadaic acid (OA)-group toxins had been confirmed to be responsible for diarrhetic shellfish poisoning (DSP) incidents due to consuming mussels *Mytilus galloprovincialis* in coastal cities near the East China Sea in May 2011. In the present study, single-cell isolations (100 cells total for each location) of *Dinophysis* were collected from the aquaculture zones of Gouqi Island and Qingchuan Bay in July and September 2013, respectively. Shellfish samples were collected over the course of a year from the aquaculture zone of Gouqi Island and tested for lipophilic toxins. Single-cell isolations of *Dinophysis* from both sampling sites were identified under the light microscope as *D. caudata*. Average quota of PTX2, the predominant toxin component in *D. caudata* isolated from the coastal waters of Gouqi Island and Qingchuan Bay, was 0.58 and 2.8 pg/cell, respectively. Trace amounts of OA and DTX1 toxins were detected in the cells of *D.caudata*. PTX2, PTX2sa,7-epi-PTX2sa, OA, and/orDTX1 toxins were found in several samples of mussels *M. galloprovincialis* and *M. coruscus* collected in the aquaculture zone of Gouqi Island from the end of May to the beginning of July 2013. PTX2, PTX2sa, and 7-epi-PTX2sa were also detected in oyster *Crassostrea gigas* during that period, but almost no OA-group toxins were present. GYM toxin was detected in almost all samples of mussel *M. coruscus*, with highest levels occurring in winter. *D. caudata* is suggested as an important source of the PTX-group toxins in the DSP event that occurred in coastal cities near the East China Sea.

P-11

Acuminolide, a new macrolactone with polycyclic ethers from the cultured *Dinophysis acuminata*, a DSP dinoflagellate species

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Dinophysis spp. has been known as dinoflagellate species producing potent shellfish toxins like OA (Okadaic acid), DTXs (dinophysis toxins), YTXs (yessotoxins) and PTXs (pectenotoxins). For the purpose of the isolation of these compounds, we cultured *D. acuminata* to a large scale. As a result, pure PTX-2, OA, DTX-1, and PTX-2 SA were obtained from the cultured *D. acuminata*. In the course of separating these toxins, we discovered a new compound showing the different NMR and MS data from the known toxins. From a combination of NMR and MS analyses, the compound was determined as a new macrolactone (M. W. = 976) consisting of sulfate group and polycyclic ethers. This compound showed no toxicity against any cell lines, unlike the isolated toxins. We are under study for further activity test. Here, we will present the isolation and structure characterization for this compound.

P-12

Isolation and characterization of a C_9-diol-ester of dinophysistoxin-1(DTX-1) from a Korean strain of the benthic dinoflagellate *Prorocentrum convacum*

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Okadaic acid (OA), dinophysistoxin-1 (DTX-1) and dinophysistoxin-3 (DTX-3) were isolated from *Prorocentrum* species and abundant esters of okadaic acid with C_7, C_8, and C_9 diol from *Prorocentrum convacum* were reported. However, to the best our knowledge, any diol-ester of DTX was not reported. Recently, during the search for bioactive compounds from large-scale cultivation of *P. concavum*, a new C_9-diol ester of DTX-1 was isolated. This organism was collected from in Jeju Island, Korea and cultured in a volume of 500L. The structure of the compound was determined by a combination of NMR spectroscopic methods and LC-MS techniques.

P-13

Time-course variation in cellular contents of PTX-2, OA, and DTX-1 in the DSP dinoflagellate *Dinophysis acuminata* during starvation

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The DSP dinoflagellate *Dinophysis acuminata* feeds on a prey ciliate *Mesodinium rubrum* to sequester chloroplasts and perform photosynthesis. To estimate the effect of starvation on the cellular contents of PTX-2, OA, and DTX-1 in a once well-fed *D. acuminata* strain, subsamples were obtained at 2d interval for LC-MS analyses. Under 20°C, 30 psu, 60 μE m^{-2} s^{-1} condition *D. acuminata* cultures were phototrophically maintained for 2 weeks. On day 7 the experimental cultures were diluted by the same volume of fresh media. The cellular contents of PTX-2, OA, and DTX-1 were at maximum during the first 6 days of our starvation experiment followed by slowly declining period. The availability of prey ciliate *M. rubrum* seems to be an important factor which supports the high cellular contents of DSP toxins in the experimental *D. acuminata* strain.

P-14

Kinetic of PTXs and DTXs in Japanese scallops *Patinopecten yessoensis* fed with the toxic dinoflagellate *Dinophysis fortii*

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The diarrhetic shellfish poisoning (DSP) toxins dinophysistoxin-1 (DTX1) and pectenotoxin-2 (PTX2) produced by toxic *Dinophysis* spp. are accumulated by the Japanese scallop *Patinopecten yessoensis*. Besides DTX1 and PTX2 several other metabolites such as PTX1, PTX3, PTX6 and 7-*O*-acylDTX1 (DTX3) are accumulated in scallops. To elucidate the toxin dynamics scallops were fed with the toxic dinoflagellate *Dinophysis fortii*. We analysed the accumulation and conversion kinetics of PTXs and DTXs in the scallops.
Four non-toxic scallops (shell length: 8.85 cm ± 0.39) were collected at Funka Bay Hokkaido in January and were acclimated for 24 days in sand filtered seawater at 11-14°C. Three individual scallops were separately exposed to cultured *D. fortii* for 4 days in 500 mL static sand filtered seawater in a 1000 mL beaker. The number of *D. fortii* cells consumed by the each scallop was about 780,000. The total amounts of PTX2 and DTX1 taken in by the individual scallops were 47.6µg and 24.1µg, respectively. Individual tissues (midgut gland, adductor muscle, gill, gonad, mantle, the others) and cultured seawater were subjected to toxin analyses. PTX1,2,3,6, DTX1, and 7-*O*-16:0-DTX1 were analysed by LC/MS/MS. Toxins were exclusively accumulated in in the midgut gland. The dominant PTXs and DTXs detected in the midgut gland of scallops were PTX6 and 16:0-DTX1, respectively. In contrast to the midgut gland, PTX2 and DTX1 were dominant in other tissues. The accumulation rates of PTXs and DTXs in individual scallop were 20-40% and 5-16%, respectively.

P-15

Detection of marine and freshwater biotoxins in Australia

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Marine and freshwater biotoxins which are produced by microalgae had been of real risk to environment and aquaculture. These biotoxins can be accumulated in shellfish or fish and cause severe human poisoning after consumption of the contaminated seafood. The shellfish quality assurance programme in Australia was initially established using phytoplankton monitoring and mouse bioassay; however, as the slow response and unacceptable method limits, the aquaculture industry and the food safety authority opted for the faster and more sensitive chemical analysis methods since 2012. Now, our laboratory have established multi toxins methods and is able to provide comprehensive routine monitoring of a wide range of toxins including PSP, ASP, DSP, yessotoxins and freshwater cyanotoxins. More than 10 PSP toxins can be quantitatively and qualitatively analysed using modified pre-column oxidation method using HPLC-FLD [1,2]. Two analytical methods were developed to determine marine lipophilic, DSP, ASP[3,4] and freshwater toxins respectively using UPLC-MSMS as a detection technique. For key toxins fortification levels were between 25-250 µg kg^{-1} in oyster, mussel, abalone and scallop. The recoveries were 92–125% for PSP toxins, 107-124% for domoic acid, 88-118% for okadaic acid, dinophysistoxin 1 and 2, 103-108% for pectenotoxin-2, 98-102% for yessotoxins, 102-110% for azaspiracids, 104-108% for gymnodimine and spirolide-1, 93-115% for cyanotoxins (microcystin-LR/RR, nodularin and cylindrospermopsin). The repeatability and reproducibility, relative standard deviations (RSDs), were < 20%. The limits of reporting are 25-50 µg kg^{-1} saxitoxin equivalents for PSP toxins and 25 µg kg^{-1} for other toxins except microcystins.

P-16

Uptake and depuration of free and covalently bound microcystins in edible clam *Corbicula leana* exposed to cyanobacterial crude extract

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Filterfeeders, such as bivalves, are highly affected during toxic cyanobacterial blooms or after bloom decay as they are non-selective and may use cyanobacteria as main food source. In the present study, the uptake and depuration of free and covalently bound microcystins (Co-MC) in edible clam *Corbicula leana* were investigated under laboratory conditions. Clams were exposed to a cyanobacterial crude extract (CRE) containing 400 µg MC-LR_{eq} L^{-1} for 10 days, followed by 5 days transferred to toxin-free water. Free MC concentrations in clam tissue and incubation water were monitored by *high performance liquid chromatography (*HPLC), whereas Co-MC was quantified via 2-methyl-3-methoxy-4-phenylbutyric acid (*MMPB*), a decomposition product of *MC* by *gas chromatography–mass spectrometry* (*GC-MS*). The results showed that free MC was taken up quickly and excreted rapidly during exposure and depuration periods. No free MC was detected after 24h in the depuration period. However Co-MC was detectable until day 5 of the depuration period. Maximum concentrations of free and Co-MC were 3.4 ± 0.17 and 0.3 ± 0.003 µg g^{-1} dry weight (DW), respectively. MC concentration in the incubation water decreased gradually during the exposure period. The estimated daily intake (EDI) of total MC concentration in the clam was far beyond the WHO's provisional tolerable daily intake (TDI) of 0.04 µg kg^{-1} day^{-1}, suggesting that consumption of clams collected during cyanobacterial blooms or immediately after blooms should definitely be avoided.

P-17

An individual based model on the toxin uptake and removal in a bivalve population caused by the vegetative cells and resting cysts of a bloom of toxin producing plankton

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HABs (Harmful Algal Blooms) and PSP (Paralytic Shellfish Poisoning) events are recurrent problems in many parts of the Philippines. The need for improved monitoring, research and prediction tools (i.e. ecological models) is thus constantly in demand. In order to simulate toxin dynamics in a population of bivalves, a hydrodynamic model coupled to an individual based model (IBM) was developed. The hydrodynamic model provided the environment (i.e. water velocities, salinity, temperature and light) for the agents of the IBM. Agents included bivalves and toxin producing plankton (TPP) represented as vegetative cells and resting cysts. Bivalves interact with TPP by ingesting the cysts and cells, and accumulating the toxin. The model was run for a year with one bloom of TPP occurring early in the model run. Two different model methods were implemented in separate model scenarios. The first model scenario implemented a method where bivalves ingested cells while the other scenario implemented a method where bivalves ingest both cells and cysts. A separate model scenario was seeded without bivalves. Data from different runs were compared in order to give insight on the role of cells, cysts and bivalves in HABs and PSP events. Comparisons of data from different model scenarios were compared with available field data. This model will be used to further investigate HAB dynamics and interactions with bivalves and their toxicities.

P-18

Transient isotachophoresis-capillary zone electrophoresis for the analysis of paralytic shellfish toxins in mussel sample

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Accumulation of paralytic shellfish toxins (PSTs) in contaminated shellfish is a serious health risk making early detection important to improve shellfish safety and biotoxin management. Capillary electrophoresis (CE) has been proven as a high resolution separation technique compatible with miniaturization, making it an attractive choice in the development of portable instrumentation for early, on-site detection of PSTs. In this work, capillary zone electrophoresis (CZE) with capacitively coupled contactless conductivity detector (C^4D) was compared with UV detection because of its small footprint. To improve sensitivity and deal with the high conductivity sample matrix, counter-flow transient isotachophoresis (tITP) was used, exploiting the high sodium levels in the matrix as leading ion while L-alanine was used as the background electrolyte (BGE) and terminating electrolyte (TE). Careful optimization of the injected sample volume and duration of the counter-flow resulted in limit of detections (LODs) ranging from 74.2-1020 ng/mL for tITP-CZE-C^4D and 141-461 ng/mL for tITP-CZE-UV, an 8-97 fold over reduction compared to conventional CZE. The LODs were adequate for the analysis of PSTs in shellfish samples close to the regulatory limit (800 ng/mL). The developed method was applied to the analysis of a contaminated mussel sample and validated against results from Association of Official Analytical Chemists (AOAC)-approved method for PSTs analysis using pre-column oxidation of the sample prior to analysis by high performance liquid chromatography (HPLC) with fluorescence detection (FLD). The method presented has potential for incorporation into field-deployable devices for the early detection of PSTs on-site.

P-19

Cyanopeptide profile of the toxic cyanobacterium *Sphaerospermopsis torques-reginae*

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The *Sphaerospermopsis torques-reginae* is a freshwater filamentosous cyanobacterium, previously reported as responsible for several toxic blooms in water bodies in Northeastern Brazil. This cyanobacterium produces anatoxin-a(S), a potent irreversible inhibitor of acetylcholinesterase. Apart from this toxin little attention has been placed in the production of other secondary metabolites by this genus. In this work, the cyanopeptide profile of the toxic cyanobacterium *S. torques-reginae*, isolated from the Tapacurá reservoir in Brazil, was investigated by liquid chromatography coupled to high-resolution mass spectrometry (LC-QTOF-MS/MS). These analyses revealed the presence of several known compounds belonging to anabaenopeptins and spumigins families, along with two putative novel cyclopeptides. Planar structures for these compounds were proposed based on the accurate mass of both molecular ion and MS/MS fragments, together with other chemical characteristic such as UV-vis spectrum. Further analyses are being carried out in our laboratory to confirm these structures.

P-20

The sugar kelp *Saccharina latissima* is a potential source of the emerging toxin, Pinnatoxin-G, in cold waters.

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The presence of cyclic imine toxin, pinnatoxin-G, was unambiguously identified in the sugar kelp *Saccharina latissima* from Norway by liquid chromatography coupled to mass spectrometry analysis, running under several operation modes. Enhanced product ion scans acquired at 30, 50, 70 and 90 eV of collision energies from the precursor $[M+H]^+$ of PnTX-G at *m/z* 694.5, matched with those obtained for the analysis of PnTX-G reference solution. Quantification was performed with the mass spectrometer operating in multiple reaction monitoring mode, with selected transitions *m/z* 694.5 > 676.4 or 694.5 > 164.5. Six-level calibration curves between 0.19-38 µg PnTX-G·mL^{-1} showed good intra-batch performance: linear adjustment (r^2) between 0.9961-0.9999 and slope shift between 0.5- 5.7% among subsequent calibration curves. The concentration of PnTX-G in *S. latissima* under these conditions was estimated of 5.1 ± 0.4 µg·kg^{-1}. This finding strengthens the evidenced of a wide latitudinal distribution of pinnatoxins, and it suggests that kelp or seaweeds can be a potential ecological niche for benthic dinoflagellates producers of pinnatoxins in cold waters. Assessment on how food processing may affect to the levels of this toxin in manufactured food products containing kelp-based ingredients should be further investigated.

P-21

Novel Ovatoxin-g and putative palytoxin from *Ostreopsis cf. ovata* (NW Mediterranean Sea): gaining structural information through high resolution mass spectrometry

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Blooms of the benthic dinoflagellate *Ostreopsis* cf. *ovata* are a concern in the Mediterranean Sea, since it produces a wide range of palytoxin-like compounds listed among the most potent marine toxins. This study focused on two analogs of palytoxin found in cultures of *Ostreopsis* cf. *ovata* strain IRTA-SMM-11-10 isolated from the south of Catalonia (NW Mediterranean Sea) in August, 2011. Our strain produced two novel compounds, ovatoxin-g and an isomer of palytoxin whose structures had not been elucidated yet. Insufficient quantity of these compounds impeded a full NMR-based structural elucidation, thus we studied their structure through Liquid Chromatography Electro Spray Ionization High Resolution Mass Spectrometry (LC-ESI-HRMS) in positive ion mode, following the LC-ESI-$HRMS^n$ approach developed by Ciminiello et al. 2012. Under the used MS conditions, the molecules underwent fragmentation at many sites of their backbone and a large number of diagnostic fragment ions were identified. As a result, ovatoxin-g was tentatively identified as 46-deoxy-ovatoxin-a, while the novel isomer of palytoxin would have two additional hydroxyl groups (one in the region C1-C8 and one at C42) and two hydroxyl lacking (one at C17 and one in the region C53-C79), compared to palytoxin standard. Ovatoxin-g and putative palytoxin were the least abundant palytoxin-like compounds (0.8% and 0.4%) in a toxin profile dominated by ovatoxin-a (53%). Ovatoxin-b to –e (30%, 4%, 6% and 7%) were also present, while production of ovatoxin-f was not observed. Strain IRTA-SMM-11-10 is still under investigation to confirm the presence of other potentially new palytoxin congeners.

P-22

Complex toxin profile of French Mediterranean O*streopsis* cf. *Ovata* strains and ovatoxins prepurification

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In French Mediterranean coastal waters, *Ostreopsis* cf. *ovata* produces, in addition to putative palytoxin (p-PLTX), mainly ovatoxins, which can accumulate in marine organisms. However, purified ovatoxins are not widely available and their toxicity remains poorly characterized. Live *Ostreopsis* cells were collected at Villefranche-sur-Mer and highly toxic cultures were obtained following optimization of environmental conditions. Pellets of cultured *Ostreopsis* cells were used to develop a purification protocol of OVTXs. LC-MS/MS analysis of *Ostreopsis* cells showed the same toxin profile both *in situ* and in laboratory culture, with ovatoxin-a (OVTX-a) being the most abundant analog (~50%), followed by OVTX-b (~15%), p-PLTX (12%), OVTX-d (8%), OVTX-c (5%) and OVTX-e (4%). Toxin profiles in *Ostreopsis* cells *in situ* were conserved while concentrations decreased with increasing depth.
Ostreopsis cf. *ovata* produced up to 2 g of biomass per L of culture, with a maximum concentration of 300 pg PLTX equivalent cell^{-1}. Methanolic extracts were purified using different techniques including liquid-liquid partition or size exclusion. Among these methods, open-column chromatography using LH-20 gel provided elimination of a large fraction of undesirable compounds (93%) with a comparatively high yield of ovatoxins (85%) in the fraction of interest. However, despite the increase in toxin purity in this fraction (by 13-fold), additional purification steps are currently in progress using the preparative MS-guided HPLC.

P-23

Toxicity in *Ostreopsis* strains from northeast Brazilian coast

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This study describes the detection and identification of seven analogues of palytoxin in *Ostreopsis* strains from different Bahia coast areas, northeast Brazil, both by hemolysis neutralization assays and LC-TOF-MS/MS. The strains were identified as *O.* cf. *ovata* (strains 12, 13, 37), *O. labens* and *O. siamensis*. Cells were collected in the exponential phase by filtration and extracted using methanol. Only the extracts of *O*. cf *ovata* (strains 13, 37) and of *O. labens* were analyzed by hemolytic assays, which showed positive results. The mass spectra obtained on TripleTOF enabled the identification of palytoxin and six analogues (PLTX, putative-PLTX, OVTX-a, OVTX -b, OVTX -c, OVTX –d, OVTX -e). All toxins were detected in *O. labens* and in *O.* cf. *ovata* (strains 13, 37). *O. siamensis* showed only OVTX –d/e and OVTX -b/c were not detected in the *O*. cf. *ovata* 12 strain.

P-24

Azaspiracid production by an *Azadinium* like flagellate isolated from a shallow mixosohaline mangrove system at southern Brazilian coast

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Azaspiracids were first time found in mussel samples from South Brazil in 2009. Until at the present time, no any organism has being identified as AZA producer. In this study we report the azaspiracids production by a small sized organism (11-16 µm length; 7-10 µm wide), collected in September 2013 at lower part of Canal do Linguado ($26^0$27'17''S 48^o36'06''W). This is a shallow mangrove region that belongs to the major Babitonga Estuary system, at the coast of Santa Catarina. The flagellated cell was maintained in f/2 culture media, at 31 psu and 22^oC, in a 14/10h (light/dark) cycle, and 70 to 100 µM photons m^2S^{-1} of light intensity. The cells presented a typical *Azadinium* like shape and the conspicuous swimming behavior consisting of rapid "jumps" followed by normal swimming. The cells were harvested during the lag phase and concentrated onto glass fiber filter for further toxin analysis. The filters were extracted on 100% methanol and analyzed by high resolution and mass accuracy electrospray TOFMS>IDA>MS/MS on an AB Sciex TripleTOF 5600 system. The acquisition mass ranges were set to *m/z* 500-3000 (TOFMS) and *m/z* 100-3000 (MS/MS). The spectra obtained on TripleTOF enabled identification of previously known analogs of azaspiracids. Were detected 9 azaspiracids analogues in 3 replicas filters. These results confirm the presence of AZA toxins in the Brazilian coast. Furthers studies are in course to elucidate the taxonomy of the producing organism and if this is the only source for AZA toxins potentially found in seafood in the region.

P-25

The chemistry of *Prymnesium parvum* and related species

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Prymnesium parvum has been associated with massive fish kills in brackish and in more saline waters, which can have dramatic economics consequences for the fishing industry and especially for farmed fish. The ichthyotoxic compounds responsible for this have previously been assigned to large molecular weight polyethers called prymnesins, and more recently also to variety of fatty acid amides and different monogalactomono- and diacylglycerides.
In the larger Danish strategic research project, "Harmful algal blooms and fish kills", we are currently using *P. parvum* as a model organism to link algal compounds to observed fish mortality. By using UHPLC-*state-of-the-art* High Resolution qTOF-MS (< 1 ppm in mass accuracy) and NMR we have found that one out of 10 tested strains is likely producing the original prymnesin 2 toxin. In addition our results indicate that a Danish strain produces another yet unidentified prymnesin like compound.
All algal extracts are fractionated by Explorative-Solid-Phase Extraction and tested (with pure compounds) for bioactivity against: i) gill cell lines; ii) *Artemia salina* and; iii) the microalgae *Teleaulax acuta*. So far our results indicate that prymesins are responsible for the observed toxicities, and that fatty acid amides are not true algal products. In the near future we will be comparing the chemistry of *P. parvum* with its related species *Chrysochromolina sp.*

P-26

Molecular characters and toxin profiles of Chinese *Gymnodinium catenatum* strains

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Gymnodinium catenatum has been reported to cause massive blooms in the South China Sea and Yellow Sea of China, however, detailed examinations of Chinese *G. catenatum* are limited. Here we established eleven strains of *G. catenatum* by incubating microreticulate cysts whose diameter ranged from 45 to 55 µm. This kind of cyst was encountered in the South China Sea and Yellow Sea of China and yielded chains of 4-8 cells. All these strains were conservative in terms of large subunit ribosomal DNA (LSU rDNA) and internal transcribed spacer region (ITS) sequences. In addition, they shared identical ITS sequences with those from Europe, South America, South-east Asia, thus can be classified as C-gene type and global populations. Five strains from the Yellow Sea (1 from Dalian and Qingdao each and 3 from Lianyungang) and 1 strain from the South China Sea (Fangchenggang) were tested for production of paralytic shellfish poisoning toxins. All strains produced abundant C3/4 and C1/2. Most of the strains produced abundant GTXs, which is a rare trait in *G. catenatum*. The 11-sulphated benzoate analogues -GC-GTXs- were much more abundant than the non-11-sulphated GC-STXs, clearly different from Portuguese/Mexican strains tested by same HPLC method. The strain from Dalian (close to Korea) differed from other Chinese strains in having a high proportion of GCs-sulfo in relation to GC-GTXs. Our results suggest that Chinese *Gymnodinium catenatum* might represent a unique population.

P-27

Chemotaxonomic studies of *Alexandrium* and *Karlodinium* using high resolution mass spectrometry

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Species determination of dinoflagellates can often be difficult and time consuming using conventional techniques (electron microscopy, phenotypic, developing specific genes/primers). Analysis of the metabolites produced by species of dinoflagellates may have the potential to identify bioactive compounds only found in active strains, but also aid species identification, helping to improve accuracy and potentially decreasing the time needed for identification. Due to the chemical complexity from high amounts of ubiquitous compounds (chlorophyll, lipids etc.) and low amounts secondary metabolites, NMR spectrometry is not a practical method for screening dinoflagellates extracts, thus the choice of ultra-high performance liquid chromatography (UHPLC) coupled with high resolution mass spectrometry (HRMS). Results from which can be obtained from biomass grown in the 50 mL scale, with compounds of low abundance still being detectable. For this we have found the aggressive dereplication approach[2] efficient, and here we use an improved version with qualifier ions of both described compounds and likely derivatives resulting in increased efficiencies.
Data from the two genera were also analyzed by multivariate methods to identify minor but important components that were then feed into the aggressive dereplication database for recursive data analysis. This poster will describe both the data analysis approaches and the results for screening of +40 strains.

P-28 ST-09

Identification of 12 β-deoxydecarbamoylsaxitoxin in the toxic marine dinoflagellate

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In the search of saxitoxin (STX) analogues using HILIC-ESI-Q-Tof-MS, some peaks were found on the chromatogram (*m/z* 241.1408 ± 0.01) of the toxic sub-clone of marine dinoflagellate, *Alexandrium tamarense*, Axat-2, while no peak was detected on that of the non-toxic sub-clone, UAT-014-009. These compounds (molecular formula: $C_9H_{17}N_6O_2$) were compared with 12α and β-deoxydecarbamoylsaxitoxin (deoxy-dcSTX) which were chemically derivatized from dcSTX by the reduction with sodium borohydride under the conditions reported by Koehn *et al*. The HILIC-ESI-Q-Tof -MSMS and the highly sensitive HPLC with post-column fluorescent derivatization confirmed the presence of 12β-deoxy-dcSTX in the toxic strain of *A. tamarense* which contained C2 and GTX4 as main toxins. Moreover, *A. catenella* and unidentified *Alexandrium* sp. also contained 12β-deoxy-dcSTX along with C2 and GTXs. Previously, 12β-deoxy-dcSTX (= LwTX4) was isolated from the freshwater cyanobacterium *Lyngbya wollei* which possess unique set of STX analogues. This is the first evidence of its presence in the marine dinoflagellates.

P-29

Update on certified reference materials available from the NRC Biotoxin Metrology program

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Routine monitoring of shellfish for algal biotoxins prior to harvest is essential for consumer safety and is necessary for international trade. In addition to seafood, toxins of cyanbacterial origin pose risks for the use of freshwater supplies. Accurate calibration standards as well as matrix certified reference materials (CRMs) are essential for method development and validation. The National Research Council's (NRC) metrology program produces CRMs for marine and freshwater toxins under a rigorous quality system.
Marine toxins can be divided into two general categories: hydrophilic and lipophilic. The hydrophilic toxins include the domoic acid and saxitoxin groups. The lipophilic toxins include the okadaic acid group, pectenotoxins, azaspiracids, yessotoxins, and the cyclic imines. Fresh and brackish water toxins comprise compounds primarily from the microcystin, cylindrospermopsin and anatoxin groups.
This poster will provide an update on the biotoxin CRMs available from the National Research Council covering both calibration solution and matrix CRMs. The high purity solution CRMs are critical for instrument calibration and general research applications, while the shellfish tissue matrix CRMs are important for verifying complete analytical methods, from sample preparation through to final data analysis. Planned future CRM developments will also be shown.

P-30

Production of standard materials of paralytic shellfish toxins by batch cultures of *Alexandrium tamrense* and *Gymnodinium catenatum*

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High performance liquid chromatography (HPLC) with fluorescent detection and HPLC mass spectrometry are one of the promising alternatives to animal bioassay for paralytic shellfish toxins (PST). The supply of certified standards for the methods is a critical issue for the methods. For the production of the standards from cultured cell of causative dinoflagellates we screened the strains which have preferable toxin composition. Over one hundred strains of *Alexandrium tamarense* and *Gymnodinium catenatum* were newly isolated, and the strains rich in gonyautoxin (GTX) 1, GTX4, GTX5 and GTX6 were selected. Among these strains of *A. tamarense* we found the strain rich in GTX1 and GTX4 over 80% of the total toxin and have no N21-sulfocarbamoyl gonyautoxin 2 (C1) and N21-sulfocarbamoyl gonyautoxin 3 (C2). The total of GTX5 and GTX6 accounted for over 60% of the total toxin in several isolates of *G. catenatum*. We already had the strain rich in C1 and C2 and other toxin analogues necessary for PST monitoring of bivalves in Japan can be prepared by chemical transformation of the 6 toxin analogues from the cultured cells. The method of large batch culture (10 L) for *A. tamarense* and *G. catenatum* were also developed using round-bottomed reservoir with aeration. Using this method *A. tamrense* reached a density over 40,000 cells/mL in 3 weeks. Also chain forming *G. catenatum* grew well and reached a density about 8,000 cells/mL in adequate volume of aeration.

P-31

Emergent lipophilic shellfish toxins in the Magellan region (47-55 ºS), Chile

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The knowledge about lipophilic shellfish toxins (LST) and its primary source is still scarce in Chile. Particularly in the Magellan region the LST have been overshadowed by the high incidence of paralytic shellfish poison (PSP) associated to *Alexandrium catenella*. However, in the last two years the LST levels have become a problem since closed areas for shellfish harvesting have increased in its annual frequency.
During the summer 2012-2013 an intense dinoflagellate bloom occurred, among which *Dinophysis acuminata* and *Protoceratium reticulatum* highlighted. The high relative abundance (RA) of these microalga remained between December 2012 and February 2013 allowing shellfish and phytoplankton collections (net hauls from 20 m depth) to establish the lipophilic toxins profiles by LC-MS/MS.
PTX2 was detected in a phytoplankton with a high *D. acuminata* RA (3 to 9, regular to mega abundant, respectively) while only traces of PTX2 were detected from picked cells. YTXs were detected from picked and culture cells of *P. reticulatum* and also in the phytoplankton with a high RA for this species (1 to 6, rare to extremely abundant, respectively).
The results indicate that of the four toxins detected, three may be detected simultaneously in molluscs. The highest toxin concentration in shellfish was associated with the dominance of toxins detected in the phytoplankton when *D. acuminata* and *P. reticulatum* had a high RA. The primary source of DTX1 and SPX1 remains still unclear.

P-32

First report of PSP, DSP, ASP and NSP toxins from Qatari Waters (Arabian Gulf)

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Harmful algae bloom is a natural phenomenon due to increase of algae cell density in water column that subsequently causes deleterious effects to natural environments as well as mankind. HABs in the Qatari waters occurred when a particular group of phytoplankton cells proliferate in the eutrophied semi-enclosed water body. In this study, HABs composition in the Qatari waters was determined by light and scanning electron microscope (SEM). Plankton samples were collected by a 20-micron plankton net haul in the Qatari waters during Oct. 2012 and Nov. 2013. The collected samples were divided into two jars, one for culture, toxicity testing and the other for identification. Samples underwent fixation and serial dehydration. Samples were observed under SEM. A total of 20 HABs were identified with 15 species known to be associated with HABs events and a total of 15 toxic species were identified with PSP dominancy. The occurrence of fish-killing armored dinoflagellate, *Vulcanodinium rugosum* was reported for the first time from the Arabian Gulf and first report of a noval pinnatoxin and portimine. The presence of 12 major algae toxins and other potentially HABs in the Qatari Waters should be taken seriously by respective authorities in future.

P-33

Phosphorus depletion and enrichment effects on cell growth, toxicity and protein expression in DSP toxin producing *Prorocentrum hoffmannianum*

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Prorocentrum hoffmannianum is one of the diarrheic shellfish poisoning (DSP) toxins producers. There are several studies on the effects of phosphorus on the growth and toxicity of *Prorocentrum* species, but very little is known about changes inside the cells at the molecular level. Protein expression analysis of the cells grown with or without phosphorus supplements has the potential to disclose important cellular pathways. In this study, the growth, cellular toxicity and protein expression of two *P. hoffmannianum* strains (CCMP683 and CCMP2804) under two phosphorus conditions, either enriched with phosphorus (P) or starved of P (P-depleted condition) were investigated. Growth of both strains was inhibited in the P-depleted condition and cell numbers remained at around 3000 cells/mL throughout the growth cycle. The growth rates of both strains under normal and P-enriched conditions were highly similar. The maximum cell density (53167 cells/mL) of the P- enriched cultures of CCMP2804 was higher than that of the normal cultures (at which the maximum cell density was 39022 cells/mL). However, the maximum cell density of CCMP683 under P-enriched condition reduced from 34967 cells/mL to 23578 cells/mL. These results indicate the variability exist in the *P. hoffmannianum* strains in response to the effect of phosphorus. Toxicity of both strains under the two different phosphorus conditions was detected by using UPLC-FLD method. Interestingly, no OA, DTX-1and DTX-2 was detected in CCMP683 regardless to the phosphorus conditions. On the other hand, CCMP2804 under the P-depleted condition produced the highest amount of okadaic acid (67.03 pg/cell). Protein expression of the both stains under the normal and P-depleted conditions was analysed by using two dimensional gel electrophoresis (2-DE). In total 54 differentially expressed protein spots (at least 4 folds difference) were found in the comparison of 2-DE profiles between the two strains under normal condition, as well as the P-depleted condition. These differentially expressed proteins potentially involved in the cellular processes that may regulate the growth and toxin production in response to different phosphorus conditions.

P-34

Short-term response, uptake and depuration of toxin from *Perna viridis* fed with the dinoflagellate, *Alexandrium tamarense*

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Shellfish are organisms prone to concentrating toxin produced by harmful algal species. These shellfish are an important source of protein in many parts of the Philippines and are heavily harvested sometimes even when there are bans in effect. This study focused on the short-term response, uptake and depuration of the Southeast Asian green-lipped mussel *Perna viridis* fed with the toxic dinoflagellate *Alexandrium tamarense*. Specifically, it attempted to understand the ability of mussels to eliminate toxin from its system within a short timeframe and its role in transforming toxin to different types or forms. The experiment was conducted within a 24-hour period with a series of subsampling every four hours. For each aquarium, two mussels were isolated for every sampling period. Toxin extraction for the first sample was done with hydrochloric acid while acetic acid was used on the other samples. Only gut samples were analysed for this study. Results showed no significant difference between hydrochloric acid and acetic acid. Three standards were used for toxin analysis: Saxitoxin (STX), Gonyautoxin (GTX), and Neo-saxitoxin (NSTX). Based on potency, STX has the highest potency followed by NSTX and lastly by GTX (dominant form of toxin for *Alexandrium tamarense*). Results showed that during the first part of the grazing experiment, GTX was the dominant type. After 12 hours, peaks for NSTX readings appeared, pointing to the ability of the mussels to convert a less potent toxin form to its more potent variant which would be more harmful to humans.

P-35

Studies on lipophilic toxins in hydrobiological organisms from southern Chile

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The present work shows the identification of lipophilic toxins associated with DSP-toxins identified in various hydrobiological organisms such as *Mytilus chilensis*, *Aulacomya ater*, *Choromytilus choros*, *Venus antiqua*, *Gari solida*, *Tagelus dombeii*, *Thais chocolata*, *Concholepas concholepas* and *Fissurella* spp, as well as the distribution level of toxins in the different tissues. Species were collected from Islas Huichas, south of Chile. Liquid chromatography with mass spectrometry (LC-MS) was used for identification of DSP toxins. The detected concentrations were variable and dependent on the habitat of the species. Maximum detected levels corresponded to 60.6 μg OA equiv kg^{-1} in *M. chilensis*, 155.7 μg OA equiv kg^{-1} in *A. ater*, 10.1 μg OA equiv kg^{-1} in *C. choros*, 107.1 μg OA equiv kg^{-1} in *V. antiqua*, 58.4 μg OA equiv kg^{-1} in *G. solida*, 71.8 μg OA equiv kg^{-1} in *T. dombeii*, 411.4 μg OA equiv kg^{-1} in *C. conchalepas*, 67.2 μg OA equiv kg^{-1} in *T. chocolata* and 43.7 μg OA equiv kg^{-1} in *Fisurella* spp. The anatomical distribution of toxins evidences that species of rock strata (A. ater) showed high levels in hepatopancreas>adductor muscle>>mantle>gills; sandy bottom species (*G. solida*) showed a decreasing distribution of hepatopancreas > mantle >> adductor muscle > gills and toxicity (97.4%) of carnivorous species (*C. Concholepas*) was only detected in the foot, represented by a 95.5% for OA and 4.5% for DTX-1. The significance of this study was to determine the high variability and distribution of toxins, which shows the high variables in defining a toxic profile.

P-36 ST-10

In vitro studies on intestinal absorption and toxic effects of pinnatoxins (A and G) and of *Vulcanodinium rugosum* extracts

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Recently, *Vulcanodinium rugosum* has been described in a French Mediterranean lagoon. Cultures of the dinoflagellate as well as shellfish sampling indicated that pinnatoxin congeners, toxins of the cyclic imine group, are produced and may accumulate within the food web. Due to public health concern, since humans may be exposed to these compounds through oral intake, kinetics and toxicological data are required for these toxins as well as for *V. rugosum* extracts. In this study, pinnatoxins A and G as well as different purified extracts of *V. rugosum* have been tested *in vitro*. First, experiments on the human Caco2 cell model mimicking the intestinal epithelium were carried out to evaluate the intestinal barrier crossing. The quantification of pinnatoxin A and G was performed by ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Secondly, toxic effects including cytotoxicity, apoptosis, proliferation and inflammation were measured on Caco2 and Neuro2A cells based on viability tests, ELISA and immunostaining quantification using an innovative High Content Analysis approach.
Our results showed that pinnatoxins can be moderately absorbed across the Caco2 cell monolayer with an apparent permeability coefficient (P_{app}) depending on the time of exposure (from 2 to 24 hrs) and the state of purification of the *V. rugosum* extract. While no toxic effects were reported on both cell lines with pure pinnatoxins, some toxic effects have been detected with *V. rugosum* extracts. Investigation of the toxic compounds inducing such effects produced by *V. rugosum* must be carried out to provide further data for risk assessment.

P-37

Assessing the neurotoxic effects of *Ostreopsis* cf. *ovata* by using microelectrode array based platform

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New public health risks have started being recurrent since *Ostreopsis* cf. *ovata*, a benthic dinoflagellate widespread in tropical seas, has colonized several areas of the Mediterranean coasts. *Ostreopsis* species produce a number of palytoxin-like compounds, termed ovatoxins, along with trace amounts of putative palytoxin as the causative agents of the *O. ovata* related human intoxications. So far, any risk assessment for ovatoxins as well as establishment of their molecular target have not yet been elucidated. Toxicity reports are usually referred to the concentrations of *Ostreopsis* cells recorded in seawater, but this data is not *per se* predictive for human risk, since dinoflagellates do not always produce the same amount of different toxins. Furthermore, *Ostreopsis* cell debris can be present in the marine aerosol and their contribution to the effects on human health cannot be excluded. In order to assess the neurotoxic potential of the *O.* cf. *ovata*, we performed electrophysiological measurements on neuronal networks coupled to microelectrode arrays. Toxicity of sonicated cells and filtrate growth medium at the end of stationary algae growth phase was evaluated. The integrated analysis of specific parameters related to the spontaneous electrical activity showed that sonicated cells were the most effective with maximal inhibition around 70% gained from 2 cells/mL concentration. Differently, a maximal inhibition around 20% was obtained with the filtrated growth medium corresponding to 7 cells/mL concentration. The results show that the platform based on microelectrode arrays is a fast and highly sensitive screening system for detecting and evaluating the neurotoxicity of environmental biotoxins.

P-38

Domoic acid disrupts neuronal activity and signalling patterns in organotypic brain slice cultures

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Domoic acid, produced by *Pseudonitzschia* spp., poses neurological health risks to marine wildlife and humans during harmful algal blooms. As a glutamate analogue, domoic acid binds to neuronal receptors and triggers excitatory signalling. The development of increasingly advanced multi-electrode arrays offers the ability to record the firing activity of many neurons simultaneously. We used a novel 512-electrode array to assess domoic acid's effect on neuronal network activity and firing patterns in organotypic mouse brain slice cultures. The spatial coverage of our array is enough to record from nearly all of the hippocampus – the brain region known to be damaged by acute domoic acid exposure. Cultures were maintained in the absence or presence of 0.1 µM domoic acid for 12-13 days, followed by a 90-minute recording of spontaneous electrical activity. Data from each culture were spike-sorted to identify individual electrophysiologically active neurons. We found that domoic acid exposure significantly reduced the neuronal mean firing rate, but significantly increased the burst rate (tightly clustered firing events), indicating that domoic acid-exposed neurons are far more likely to fire in a burst pattern compared to control neurons. The effective connectivity density of neural networks in exposed cultures, as measured by transfer entropy, was also significantly increased compared to control cultures. Taken together, these results demonstrate that domoic acid significantly alters neuronal firing rates and patterns, possibly due to abnormal synapse maintenance or formation. These data will shed further light on the electrophysiological changes that domoic acid exposure induces in the hippocampus.

P-39

Effect of single-dose cylindrospermopsin (cyanobacterial toxin) in the murine reproductive system

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The cyanotoxin (cyanobacterial toxin) cylindrospermopsin (CYN) is a water-soluble alkaloid that has been linked to deaths of patients at a hemodialysis clinic intravenously exposed to contaminated water. It is known that this toxin is a potent inhibitor of protein synthesis, cause severe damage in various organs and have the potential role of endocrine disruptor. Therefore, the aim of this study was to evaluate the effect of CYN in the murine reproductive system. Dose-response curves were performed with intraperitoneal doses of purified CYN (0, 16, 32, 64, 128, 160, and 256 µg of CYN/kg body weight) in females and males (129/Sv x C57/BL6) (protocol CEUA IBCCF169). Ous results indicate that females may have more efficient clearance mechanism, as they were resistant to higher doses compared to the rate mortality of the males. Gross changes in the eyes, hepatomegaly, tumor and blood in the intestine were also observed. Serum testosterone levels were significantly decreased in males injected with 32, 64, 128 µg of CYN/kg body weight. The estrous cycle of females was monitored before and after injection of 64 µg of CYN/kg body weight. Through evaluation of daily vaginal cytology an important effect, with a stop or prolongation in the cycle duration, and a significant decrease in ovarian weight were noted in females exposed to CYN. So, this toxin has been shown to importantly interfere with the reproductive system of both male and female mice. Therefore, more studies need to be conducted to understand the role of CYN as an endocrine disruptor.

P-40

Analysis of renal function in mice submitted to purified cylindrospermopsin (cyanobacterial toxin)

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Cylindrospermopsin (CYN), a cyanotoxin, is a potent inhibitor of protein synthesis and damages a variety of organs such as: liver, thymus, spleen, lungs and heart. In the kidney, CYN was previously described to cause tubular necrosis, paleness and swelling. In order to contribute to a better understanding of renal toxicity of CYN the aim of this study was to evaluate the effects of CYN on renal function of murines. For this purpose we used male balb/c of 10 weeks (protocol CEUA IBCCF170) and gave a single intraperitoneal injection in animals of 5 experimental groups of different doses: 16, 32, 64, 128 to 160 µg of CYN/Kg of body weight (n=5 per group). All animals that received the doses of 128 and 160 µg of CYN/Kg died in the first 72h after administration. At the 64 µg/kg dose, a raise in glomerular filtration rate in day 7 and a decreasing in day 14 to control level were observed. This dose also caused an increase of interstitial space and collagen deposition in the renal cortex. Proteinuria was observed only at the dose of 32 µg/kg. The results show that CYN was capable of interfering in renal function. Giving the importance of the alterations and its consequences for the maintenance of health, further studies are necessary in order to the complete understanding of mechanisms whereby CYN acts in the kidneys.

P-41

Diarrheic shellfish toxins at sublethal dose produced cell proliferation in gastric and colon epithelial cell lines

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The aim of this study was to analyse the effect of DSP toxins (Okadaic Acid, OA; and Dinophysistoxin-1, DTX-1) on the proliferation of gastric (AGS and MKN-45) and colon (Caco2) epithelial cells, the main target tissues of these toxins. We hypothesized that DSP toxins, at sublethal doses, activates multiple signalling pathways, such as ERK and AKT, through the inhibition of PP2A. To demonstrate this, we carried out curves of doses and time response against OA in AGS, MKN-45 and Caco 2 cell lines, and found an increase in the cell proliferation at sublethal doses, at 24 h or 48 h exposure. We have proved that this increased proliferation is due to an overexpression of Cyclin B, a cyclin that promotes the passage from G2 to mitosis. We have demonstrated that OA induces activation of AKT and ERK in the three cells lines, showing that OA can activate pathways involved in oncogenesis. In addition, it measured the secretion of proinflammatory cytokines as TNF-α and IL-6, detecting a significant increase due to the effect of OA and DTX-1. Therefore, DSP toxins at sublethal doses can promote cellular proliferation and activate oncogenic pathways. For these reasons we advise that consumption of seafood should be free of DSP toxins.

P-42

Toxicity assessment of *Ostreopsis* and *Gambierdiscus* using sea urchin development and fish embryo toxicity bioassays

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Toxic marine micro-algae that form harmful algal blooms can have serious human health impacts worldwide. New species are rapidly being described and their associated toxins isolated and characterised. A high level of within-species variation calls for specific testing and examination of isolates from different regions. The benthic/epiphytic dinoflagellate genera *Ostreopsis* and *Gambierdiscus* produce some of the most toxic natural known compounds, including palytoxin, ciguatoxin, and maitotoxin (and associated analogues). These toxins are associated with incurable human syndromes such as ciguatera fish poisoning through consumption of contaminated finfish. The aim of the study was to assess the toxicity of known marine biotoxins (as determined by LC-MS/MS analysis) using two bioassay models. Extracts of 12 *Ostreopsis* and *Gambierdiscus* isolates (originating from the Cook Islands, Noumea, Australia and New Zealand) were characterised using LC-MS/MS methods to determine toxin content. The extracts were tested in the sea urchin (*Evechinus chloriticus*) developmental assay and the zebrafish (*Danio rerio*) fish embryo toxicity (FET) bioassays. Urchin larval development was significantly affected by two of the five *Gambierdiscus* extracts tested and partially affected by three of the seven *Ostreopsis* extracts. The FET assay showed a toxic response to four out of five *Gambierdiscus* extracts with no response to any *Ostreopsis* extracts. These results show the potential of these two bioassays as screening tools for marine algal biotoxins.

P-43 ST-05

Evaluation of ichthyotoxicity of a harmful red-tide dinoflagellate *Cochlodinium geminatum* using an *in vitro* fish gill cell line assay

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Blooms of the well known red-tide causative agents *Cochlodinium* sp. are potential toxic dinoflagellates, which appeared frequently and globally. They have caused massive mortalities of aquatic organisms and thus leading to serious economic loss. For the past few years, blooms of *Cochlodinium geminatum* have been occurred frequently in Hong Kong coastal waters. They were usually found in the Pearl River Estuary (covered the coastal waters of Zhuhai, Macau, Shenzhen and Hong Kong). Substantial amount of death fishes were reported in Tai O, Hong Kong during a recent outbreak in August of 2011. However, the ichthtotoxicity of *Cochlodinium geminatum* is still virtually unknown. In this preliminary study, we evaluate the ichthyotoxicity of a *Cochlodinium geminatum* isolated from the Hong Kong waters by using a fish gill cell line RTgill-W1. An in-house testing protocol for using the fish gill cell line in the algal exposure experiments has been optimized and developed successfully. Briefly, exposure of fish gill cells to *Cochlodinium geminatum* culture was conducted in 48 well Falcon plates at 22 °C (±1 °C) under 12:12 hour light: dark cycle with cool white fluorescent tubes in growth chamber. The fish gill cells collected from the exposure experiments were observed under inverted microscope for morphological assessment. Cell viability was measured by using trypan blue staining and counted by using the haemacytometer. Based on the preliminary results, cell death and obvious changes of the morphology were found in the algal treated fish gill cells. These preliminary results indicate the potential ichthyotoxicity of the *Cochlodinium* cells. However, more exposure experiments by using not only the *in vitro* fish gill cell line assay, but also the *in vivo* bioassay (marine medaka) is needed to be done before conclusion can be drawn.

P-44

Adverse effects of *Karlodinium armiger* on juvenile and adult stages of the blue mussel *Mytilus edilus*

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We studied the adverse effects of exposure to the harmful dinoflagellate *Karlodinium armiger* of juvenile and adult stages of the blue mussel *Mytilus edilus* in the laboratory. We mixed early larval stages with different *K. armiger* concentrations and followed the concentrations for 29 hours. At one concentration of *K. armiger* and larvae, concentrations were followed for 6 days. Furthermore, we measured clearance rates of adult mussels incubated in a range of constant *K. armiger* cell concentrations, and in controls consisting mussels fed the cryptophyte *Rhodomonas salina*. Finally, mortality rate after 24 hours of adult mussels were measured in a range of natural bloom concentrations of *K. armiger* batch cultures. We present the results and observations of the interactions between *M. edilus* and *K. armiger* observed in the laboratory. Our results suggest that this dinoflagellate can affect both recruitment and survival of mussels in nature.

P-45

Mortality and pathologies of Japanese scallops, *Patinopecten* (*Mizuhopecten*) *yessoensis*, fed monoclonal culture of the LST-producer, *Dinophysis caudata*

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Several species of *Dinophysis,* including *D*. *caudata*, cause widespread diarrheic shellfish poisoning (DSP) in humans via consumption of shellfish that have accumulated its lipophilic shellfish toxins (LSTs). Due to difficulties in culturing *Dinophysis*, many aspects of the toxication/detoxification mechanisms of LSTs in bivalves remained intractable. The recent breakthrough in *Dinophysis* culture made it possible to assess the bioaccumulation/biotransformation kinetics of LSTs in bivalves, which was the original intent of our study with Japanese scallops fed monoclonal culture of *D*. *caudata* under controlled conditions for one week. Unexpectedly, scallops were severely affected by *D*. *caudata*.

Within 48–72 h of exposure, mortalities exceeded 60% for scallops exposed to 10^5 cells/L. Scallops exhibited altered behaviour within 12–24 h, with hypersecretion of mucus and pseudofaeces, retraction of the mantle and tentacles, reduced to suppressed escape response and response to physical stimuli, paralysis, and death. The pseudofeaces and faecal pellets contained intact, and intact and partially digested *D*. *caudata* cells, respectively, showing that scallops ingested the toxic alga but pre- and post-selectively rejected it. Similar lethargic symptoms were observed for scallops exposed to 10^4 cell/L, and no mortalities were observed within a week.

This is the first report of lethal effects of a *Dinophysis* species on a bivalve, and on a bivalve species reputed for its ability to transform LSTs into compounds of much lower toxicity without being affected. The behavioural, physiological and pathological causes underlying the toxicity of *D*. *caudata* to Japanese scallops will be presented.

P-46

Mortality on zoea stage of *Litopenaeus vannamei* (Malacostraca) exposed to *Cochlodinium polykrikoides* (Dinophyceae) and *Chattonella* spp. (Raphidophyceae)

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Cochlodinium polykrikoides and *Chattonella* spp. are responsible for HAB in Mexican coasts. These species produce toxic compounds which can be harmful to different marine organisms. Scarce information exists on the impact that these species have on early life stages of crustaceans. In this work, we evaluated the toxic effect of *Cochlodinium polykrikoides* and *Chattonella* spp. isolates from the Gulf of California on early life stages of the shrimp *Litopenaeus vannamei*, for which nauplii in stage 2 were placed (1/well) in microdilution plates. In each well, 1 mL of different cell concentrations (0.5, 3, and 6 x 10^3 cell/mL) of *Cochlodinium polykrikoides*, *Chattonella subsalsa*, *Chattonella marina* var. *marina*, *Chattonella marina* var. *ovata*, as well as *Chaetoceros calcitrans,* and *Tetraselmis suecica* as controls were added, culminating the bioassay in zoea 3 stage. Nauplii were incubated by triplicate at 23°C and 37 ups of salinity. Our results showed no effect on nauplii stage, only on zoea stage. The higher mortality (100%) was caused by strains of *C. polykrikoides* and *C. marina* var. *ovata* at concentrations of 0.5 and 3 x 10^3 cell/mL, with LT_{50} values close to 3 days. This work showed that when zoea start to feed they are highly sensitive to the exposure to *C. polykrikoides* and *Chattonella* spp. Both species caused toxic effects in the early ontogeny of crustaceans, reflected in high mortality.

P-47

Development of colloidal gold immunochromatographic assay test cards for analysis paralytic shellfish poisoning and diarrhetic shellfish poisoning

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Monoclonal antibodies against paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) were developed by using cell fusion technology. The monoclonal antibody against PSP can cross react with GTX5 (gonyautoxin5), STX (saxitoxin), dc-STX, C1 & C2 (epi-GTX8&GTX8), GTX2/3、dc-GTX2/3. Another monoclonal antibody has specific immune response to okadaic acid(OA), one of the main components of diarrhetic shellfish poisoning. After antibody purification using ammonium sulfate, antibody coated with colloidal gold, synthesized coating antigen, selected and processed NC membranes, optimized the coating solution, dilution ratio of gold labelled antibody, sample dilution ratio, coated ratio of the second antibody, colloidal gold immunochromatographic assay test cards are developed. Test cards can be visualised by the red bands and indicate the presence of paralytic shellfish poisoning (PSP) or diarrhetic shellfish poisoning (DSP). Sensitivity limitation of immune test card for analysis okadaic acid (OA) is 12 ng/mL and 5 ug/100g for shellfish samples. The sensitivity of the immune test card for paralytic shellfish poisoning (equivalent to STX) is 110 ng/mL, and 11 ug/100g for shellfish sample. Analysis can be completed within 10 minutes. Okadaic acid safety threshold of international regulation is 20 ug/100g, and 80 ug/100g for paralytic shellfish poisoning. Both of the rapid immune test cards have the advantages of rapid screening of shellfish samples containing PSP or OA. They can be used in the field, and provide results meeting international regulations. The commercial prospect of both test cards is quite good.

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Evaluation of Solid-Phase Adsorption Toxin Tracking devices for the monitoring of Ciguatera

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Ciguatera Fish Poisoning (CFP) is classically known to result from the ingestion of tropical coral reef fish contaminated with ciguatoxins (CTXs), heat-stable polyethers, mainly produced by microalgal benthic dinoflagellates belonging to the genus *Gambierdiscus*. Moreover, some species of *Gambierdiscus* are known to produce maitotoxins (MTXs) that could also be accumulated in seafood and thus contribute to the complexity of CFP clinical manifestations. Solid Phase Adsorption Toxin Tracking (SPATT), coupled with appropriate analytical methods, has been introduced as a passive sampling technique to detect toxins released into the water during algal blooms. The suitability of SPATT devices for the detection of dissolved CTXs and MTXs in seawater, and thus, for field monitoring of *Gambierdiscus* toxic blooms, was evaluated in laboratory conditions. *In vitro* exposures of SPATT bags to cultures of two strains of *Gambierdiscus,* one producing CTXs (*G. polynesiensis*) and the other producing MTXs (*G. toxicus*) was conducted. The results of toxins detection using *in vitro* cell based assays (CBA) and liquid chromatography mass spectrometry (LC-MS/MS) analysis will be presented. Furthermore, first results of the field deployment of SPATT bags in lagoons of Kaukura and Mangareva (French Polynesia), where numerous cases of CFP are reported each year, will be presented.
The use of SPATT technology as a routine monitoring tool should be very useful in the understanding of CFP and should improve its risk assessment and management programs.

P-49

Application of solid phase adsorption toxin tracking (SPATT) in the East China Sea

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Zhejiang Coast along Eastern China is one of the world's main producers of mussels (*Mytilus* spp) and other bivalves of commercial value. However, this region suffers from recurrent diarrhetic shellfish poisoning (DSP) outbreaks caused by *Dinophysis* species, and the DSP outbreaks are becoming more frequent and intense. In May 2011, a seafood-borne intoxication in Zhejiang Coast sent 200 people to the hospitals, who were diagnosed as DSP poisoning. Therefore, issues of monitoring and early warning of phycotoxins off Zhejiang Coast have been raised, and will require experienced researchers to join the effort. Understanding the distribution and dynamics of DSP in this region is a scientific and managerial challenge that requires a multidisciplinary and site-specific approach. Solid Phase Adsorption Toxin Tracking (SPATT) is a newly developed *in situ* method to monitor DSP toxins. It has been well deployed in New Zealand, Australia, Norway, Spain, Ireland, and the USA. Here, we conducted an approach to expand the application of SPATT in the East China Sea, to evaluate the applicability of SPATT for detecting okadaic acid (OA), dinophysistoxins (DTXs), pectenotoxins (PTXs), and yessotoxins (YTXs) in seawater. Nine (3 x 3) sampling sites were chosen in the coast of Gouqi Island, near the estuary of Yangtze River and Hangzhou Bay in the East China Sea. The SPATT discs (9 cm in diameter), made by 120 μm mesh that contains 9g DIAION® HP-20 resin, are suspended at a depth of 2 m and retrieved every 10 days from April to October, every 30 days in November and December, and every 15 days from January to March. Shellfish was collected locally together with the SPATT discs. After being collected, the SPATTs were rinsed twice in 500 mL MilliQ water, the resins were inserted into empty 25 mL glass syringe on a manifold, 25 mL 100% methanol was added to elute the resin at 1 mL/min flow rate, concentrated by 99% nitrogen gas, re-suspended in 5 ml 100% methanol. The extracts were analyzed by LC-MS. The mussel of 100 g flesh (triplicate) was homogenized by a meat grinder. Two grams homogenized flesh was mixed for 1 min with 9 ml 100% methanol. The supernatant were decanted into 25 mL flask after centrifugation (4000xg, 5 min). Repeated once and added 7 mL 100% methanol to complete the mark, filtered (0.22 μm) before being analyzed by LC-MS. By analyzing the toxins in mussel and SPATT, we hope to establish the relationship between shellfish and SPATT. Once the relationship is established, SPATT could be used as our monitoring tools in the local system. Further, we hope to deploy the SPATT discs at the key sites in the local area and use them as early warning tools for DSP outbreaks in the region.

P-50

Experimental assessment of synthetic resins for field monitoring of hydrophilic toxins (PST & DA) in fjords and channels of southern Chile

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In the last decade, the synthetic resins (Solid Phase Adsorption Toxin Tracking, (SPATT) for example DIAION®HP20), has been widely used as a passive adsorption sampling method of screening biotoxins, mainly lipophilic marine toxins (dinophysistoxins (DTXs), okadaic acid (OA), pectenotoxin (PTXs) and yessotoxin (YTXs)), in the natural environment. Nevertheless, there are only a few studies reporting the use of these resins for screening the hydrophilic neurotoxins Paralytic Shellfish Toxins (PST), with annual frequency in the southernmost part of Chile (Aysen and Magellan region) and the Domoic Acid (DA), with moderate frequency in Los Lagos region and occasional presence in Aysen region, high frequency on northern Chile and only traces toxins records in Magellan region. In this study, we evaluate the use of these resins for the detection of PST and DA dissolved in water, in order to assess its potential use in remote areas near to marine resources farming centers. Our results showed that the resins are capable of adsorbing both PST and DA toxins from the water in the experimental stage (pilot scale). The experimental evaluation shows it as a potentially useful tool for the early diagnosis of dissolved phycotoxins in the water column, for use on fish farming centers or productive mussel zones in remote fjord and channels areas in southern Chile. Further research is required for testing the SPATTs *in situ*.

P-51

Development of bio/ceramics membrane for controlling Harmful Algal Blooms (HABs)

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Harmful algal blooms (HABs) have made massive economic losses and marine environmental disturbances in Korea. Since 1995, it causes annual losses of $100 million to the Korean aquaculture industries. Normally, loess was scattered to control HABs in Korea, but it arises secondary contamination problems. There are many papers and patents about algicidal bacteria and chemicals in Korea but it has not been applied on practical site before due to its efficiency and safety. So, highly effective and eco-friendly method to control the HABs is urgently required to be developed. We isolated 2,000 strains of aerobic, anaerobic and facultative marine bacteria from Tong Yeong Sea water and sediment. We screened more than 1,000 bacterial strains killing *Cochlodinium polykrikoides*, about 100 strains showed >30% algicidal activity. To supply and preserve algicidal bacteria continuously, we developed some bio-ceramics fusion membrane with algicidal bacteria and multi-pore ceramics. The new multi-pore ceramic materials that we made had 57%, 60% porosity, >20% absorption rate, <1 specific gravity which can float on marine. With the value of pH 6.6, 30℃, the concentration of primary inoculated bacteria was 3.3×10^7. At 4 hours, the attachment of bacteria was 9.0×10^5 cell/cm^2, which shows the optimal condition for ceramic to absorb bacteria. With developed bio/ceramics fusion membrane, algicidal bacteria will continuously and stably be supplied in marine site. As a result, this system will be promising material for HABs removal.

P-52

Effects of physiological status on the quantification of *Heterosigma akashiwo* using Quantitative Real Time PCR (qPCR) and Sandwich Hybridization Assay (SHA)

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The development of molecular technologies has enabled rapid and specific identification and enumeration of phytoplankton species. Direct comparisons of these methods with respect to physiological status, however, are sparse. We compared quantitative real-time PCR (qPCR) and sandwich hybridization assay (SHA) for enumerating the raphidophyte *Heterosigma akashiwo* at each growth phase, over a diel cycle and under nutrient limitation. To ensure consistency between comparisons, a single cellular homogenate was generated from the same number of cells during each experiment, so that results reflect changes in nucleic acid content (RNA and DNA) at each time point or in response to environmental conditions. Results show a greater level of precision in SHA results which contributed to significant differences in RNA content per cell in several of these analyses. There was significantly greater RNA content during lag and exponential phases compared to stationary phase cultures, and a significant decrease in RNA content during the light cycle compared to cells harvested in the dark. In contrast, there were no significant differences in cell density determined by qPCR over a diel cycle or during different growth phases. Both RNA and DNA content per cell were significantly lower under N stress when compared to nutrient replete conditions. Results of this study suggest that physiological status can have an effect on qPCR and SHA analysis for determination of cell abundance. Ongoing research will evaluate the extent to which these methods are able to accurately enumerate natural populations of *H. akashiwo* under a wide range of environmental conditions.

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Quantitative polymerase chain reaction and the enumeration of brown tide algae *Aureococcus anophagefferens* in field samples in coastal waters of Qinhuangdao

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Aureococcus anophagefferens is a small pelagophyte that has caused brown tide blooms in coastal waters off Qinhuangdao in recent years, bringing about significant negative impacts on the shellfish mariculture industry. Under standard light microscopy, it is visually indistinguishable from other small algae in field samples. This is because of its extremely small size. In this study, quantitative polymerase chain reaction (qPCR) based on 18S rDNA sequences was developed and used to detect and enumerate *A. anophagefferens*. The line regression (R2=0.91) was generated based on cycle thresholds value (Ct) versus known concentrations of *A. anophagefferens*. Twenty-two field samples collected in coastal waters off Qinhuangdao were subjected to DNA extraction and then analyzed using qPCR. Results showed that *A. anophagefferens* had a wide distribution in coastal waters along Qinhuangdao. Elevated *A. anophagefferens* abundance, category 3 brown tide blooms (>200,000 cells ml-1) occurred at Dongshan Beach and Tiger-stone Beach in August in 2013. In shellfish mariculture areas along coastal waters of Qinhuangdao, 4 stations had category 3 blooms, and 6 stations had category 2 blooms (35,000-200,000 cells ml-1) in August. All stations in shellfish mariculture area had category 1 blooms (>0 to≤35,000 cells ml-1) in October. Quantitative PCR allows for detection of *A. anophagefferen*s cells at low levels in filed samples, which is essential to effective management and prediction of brown tide blooms.

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Improvement of previous qPCR methods for the quantification of *Heterosigma akashiwo* cysts

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The identification and quantification of *H. akashiwo* cysts in sediments using light microscopy are difficult due to their small size and morphology that is indistinguishable from many other cells. Here, a qPCR assay for accurate cyst identification and quantification has been developed. However, past qPCR assays for *H. akashiwo* cysts have not considered the difference in gene copy number between vegetative cells and cysts. Moreover, qPCR assays for cyst quantification have shown some interference with DNA debris in the sediment. To obtain a higher accuracy of the qPCR assay, we compared gene copy number of both cysts and vegetative cells, demonstrating that gene copies in cysts were half that in vegetative cells. Hence, we determined to construct standard curve using *H. akashiwo* cysts. To remove DNA debris in the sediment, we developed an effective method using DNA elution in low ionic strength solution. A total of 18 sediment samples were analyzed by the qPCR assay on comparing the effect of DNA debris removing treatment or non-treatment. Using a regression analysis and comparison with a direct counting method, the qPCR method showed a higher correlation when using the DNA debris removing treatment (R^2=0.72, slope=1.07, P<0.001) than non-treatment (R^2=0.60, slope=3.20, P<0.001). These results suggest that this improved qPCR method will be a powerful tool for the accurate quantification of *H. akashiwo* cysts.

P-55

Rapid detection and quantitation of microcystin-producing cyanobacteria in fresh water using real-time PCR

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Cyanobacteria (Cyano) can produce toxins that pose a serious threat to human and animal health. Microcystin is the most common toxin mainly produced by three genera of Cyano: *Microcystis*, *Anabaena* and *Planktothrix*. Current methods for detection of Cyano are time-consuming and unable to differentiate toxic from non-toxic Cyano. Therefore, development of a rapid and specific method is needed for monitoring toxin-producing Cyano in water. A real-time quantitative PCR (rt-QPCR) assay was developed and validated for detection of microcystin-producing Cyano. Genera-specific primers and probes targeting the microcystin synthetase E gene (*mcy*E*)* were used for differentiation of *Microcystis*, *Anabaena* and *Planktothrix*. The limit of detection of rt-QPCR was 50 copies/mL for the three genera *mcy*E. External standard curves were established to quantify each genera *mcy*E and no cross-reaction was observed among them. The validated rt-QPCR assay was used to detect and quantify *mcy*E gene in samples collected from Alberta lakes during the open-water season of 2012-2013. *Microcystis mcy*E was detected in 57% (644/1127) of samples, present at a high level (≥ 1.0E+05 copies/mL) in 9/45 (2012) and 4/47 lakes (2013). The rt-QPCR assay is a simple, rapid and cost-effective method with high sensitivity and specificity. In addition, this method is able to differentiate microcystin-producing genera from non-toxic Cyano. *Microcystis* is found to be dominant in Alberta lakes, but *Anabaena* and *Planktothrix* were also found in some of the lakes. The new assay provides a valuable tool for monitoring blooms and fluctuation of toxin-producing Cyano in fresh water systems.

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Cyanobacteria DNA microarray for the monitoring of Harmful Algal Bloom in Korean freshwater

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An integrated cyanobacteria DNA microarray was developed to detect representative cyanobacteria involved in harmful algal blooms of Korean freshwater such as *Anabaena*, *Aphanizomenon, Microcystis*, *Oscillatoria*, and *Phormidium*. About 100 proves with lengths of 47-mer oligonucleotides were newly designed based on the genetic information of internal transcribed spacer (ITS), RNA polymerase β subunit (*rpoB*), phycocyanin subunit A and B (*cpcBA*), ribulose-1,5-bisphosphate carboxylase and oxygenase (*rbc*), and microcystin synthetase (*mcy*) genes of the target cyanobacteria. Both type strains and lake samples with known concentrations of the target cyanobacteria were tested to confirm the specific and quantitative responses of the developed DNA microarray in the range from 1 ng to 1 μg of genomic DNAs per reaction, which were correspond from about 10^3 to 10^6 cells per reaction. From 30 freshwater samples collected 8 Korean lakes during the summer season in 2013, the levels of target cyanobacteria were evaluated with environmental parameters using the developed DNA microarray, showing that 1) genus-specific identification and semi-quantitative detection for each target cyanobacteria, 2) high levels of *Microcystis* under relatively high temperature while high levels of *Aphanizomenon* under relatively low temperature, and 3) general relationships between *mcy* and non-*mcy* proves. Finally the cyanobacteria DNA microarray developed in the present study is anticipated as a useful monitoring tool to provide integrated information of cyanobacterial risks in the harmful algal bloom of Korean freshwater.

P-57

Easy detection of multiple HAB species by nucleic acid chromatography

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Of the 5,000 species of extant marine phytoplankton, approximately 300 species can form red tides or cause shellfish poisoning, and the distribution of these species is extending all over the world. Monitoring of HAB species has been hitherto conducted by morphologically-based microscopic observations. However, as many species lack distinctive characteristics, inadequate information on which to base identification may lead to misidentification and result in an inaccurate estimation of the abundance. Therefore, molecular diagnostic techniques such as real-time qPCR, DNA microarray, and/or multiplex-PCR assay have been developed to accurately identify HAB species. Kaneka Corporation, Japan has developed a novel molecular diagnostic method utilizing nucleic acid chromatography (Kaneka DNA chromatography chip), i.e. a capture DNA probe is solid-phased on a strip. PCR amplification is conducted using primer pairs added to two different tags for visualization with colloidal gold and for capture by the solid phase DNA probe. After PCR amplification of the target regions, one drop of PCR amplicon (5 μL) was sufficient to successfully detect the target species within 5 min. This DNA chromatography chip will be widely applicable to many targets through the use of different sequence capture probes. This technology was applied to the species-specific detection of HAB species. The successful development of two different multiple-PCR assays that enabled the specific detection of 5 species in the genus *Alexandrium* and 6 noxious red tide causative species such as *Chattonella*, indicate that this method is one of the most convenient, reliable and rapid molecular tools for HAB monitoring.

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Ecologicial assessment of Ca-aminoclay to control HABs, *Cochlodinium polykrikoides* and *Chattonella marina* in natural conditions using 100 L microcosms

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Harmful algal blooms (HABs) have increasingly impacted natural ecosystems damaged coastal environments, as well as human health, caused significant economic losses to fisheries. Our approach harnesses the algicidal effects of artificial clay, Ca-aminoclay, which are comprised of a high density of primary amine groups covalently bonded by metal cation backbones and assess to suppress HABs (*Cochlodinium polykrikoides* and *Chattonella marina*) as well as ecological risk using 100 L microcosms. Positively charged colloidals of Ca-aminoclay induce cell lysis in HABs within several minutes' exposure but have negligible impact on non-harmful phytoplankton. However, Ca-aminoclay to control HABs has adverse impacts such as increase in levels of dissolved inorganic nitrogen, dissolved inorganic phosphorus, dissolved silica, and dissolved inorganic carbon and change in anoxic condition. In addition, most planktonic communities responded drastically to the presence of Ca-aminoclay: total bacterial abundances increased for the first two-three days and then decreased rapidly for the remainder of the experiment. The abundance of heterotrophic flagellates and ciliates increased rapidly in association with the increase in bacterial cells. Therefore, caution should be taken when considering the direct application of Ca-aminoclay in natural environments, even though it has the advantage of rapidly removing HABs. Also, prior to field application, microcosm or mesocosm studies are underway to gain an in-depth understanding of possible adverse environmental impacts of aminoclays. Therefore, any materials made to apply in a nature must try in mesocosm.

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Are pigments useful for identification of harmful algae?

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A review is presented on the most useful marker pigments for a range of harmful algal species, from cyanobacteria, diatoms, dinoflagellates, prymnesiophytes and a few other algal groups. In general, there are no marker pigments that are specific to harmful algal species since pigments are group-specific, not species-specific. However the presence of some of these pigments, along with knowledge of the major harmful species in a particular region, can often be used as a good indication of harmful algal blooms. In the case of dinoflagellates, among which are found several harmful species, a recent survey of sixty four species (Zapata et al. 2012) resulted in the description of six pigment-based chloroplast types, only one of which contained peridinin, the typical dinoflagellate pigment. Harmful dinoflagellate species are found within five of these six types. A mixture of harmful and non-harmful species is present in each of these types, making it difficult to use marker pigments as a tool for identification of harmful algae if no other information is available.

P-60

Development of clean-up system about fish farm pollution using bio/ceramic carrier

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Since 1980's, harmful algal bloom (HAB) has been a rising problem in south sea of Korea. Because there are many fish farms located near the coast, their sewage water contained organic component causes algal bloom. Algae damage fish in the fish farm. The efficient reduction of TN, TP, COD using bacteria was the aim of this study. In previous research, we could not show the same efficiency of nutrient reduction in the field as in the lab. Because bacteria were diffused in the marine environment, reduction of TN, TP, COD was not same as with the lab scale. To solve this problem, we tried to attach bacteria onto a ceramic carrier for growing in the marine environment. The carrier (porosity 57%) can maintain bacteria consistently and increase the efficiency of nutrient reduction. The optimum conditions for bacteria absorption to the carrier was pH 6.8, at 30℃. We isolated about 1000 bacteria strains and 3 bacteria showed high efficiency for COD, ammonia, and T-P reduction. Ammonia was uptake all in 11 hour, total phosphate removal efficiency showed up to 83%, COD removal efficiency showed up to 34%. We will develop a system applying these strains with bio/ceramic carrier for a marine environmental pollution clean-up system.

P-61 ST-04

A rapid and quantitative lateral-flow-immunoassay for *Alexandrium minutum* using superparamagnetic nanoparticles

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The dinoflagellate genus *Alexandrium* is known to produce paralytic shellfish toxins that regularly impact the shellfish industry. Morphological similarities between species make accurate identification problematic. Thus, alternative methods are urgently needed for environmental monitoring. The method should be rapid, robust, discriminative, quantitative and inexpensive. In this context, for the first time a quantitative lateral-flow-immunoassay (LFIA) using superparamagnetic nanobeads has been developed. This dipstick assay relies on two distinct monoclonal antibodies directed against surface antigens of *A. minutum* and used in a sandwich format. The capture is done by monoclonal antibody (AMI2) adsorbed onto a nitrocellulose membrane to make the test line whereas the second monoclonal antibody (AMI6) is labeled with superparamagnetic nanobeads. When the immunological reaction is finished, the strip is introduced into a cassette and analyzed by a magnetic reader (MIAtek® Magnisense). The number of magnetic particles bound to the Test line allows the deduction of the *A. minutum* concentration. Nitrocellulose membrane and test line coating was optimized. Calibration curves and the detection limit are determined using sea water spiked with known amounts of *A. minutum* cells. LFIA specificity is assessed using other dinoflagellates including other *Alexandrium* species. Subsequently, *A. minutum* concentrations in phytoplankton samples of the Penze river and the bay of Brest were determined in parallel by LFIA and the standard optical microscopy procedure. Comparison indicates a good correlation between the two methods. In conclusion, this novel and simple LFIA is capable of detecting and quantifying *A. minutum* in seawater samples in less than 30 minutes.

P-62 ST-03

Comparative analyses of Sandwich Hybridization Assay and Quantitative PCR for *Heterosigma akashiwo*: Applications to field sampling and management

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There has been a recent surge in the development of harmful algal bloom (HAB) detection and quantification technologies, yet fundamental questions about intercalibrating methods remain unanswered. Thus, the HAB community identified the need for cross-validation studies as a critical priority. We report findings from an ongoing NOAA-MERHAB funded program to critically compare two molecular approaches: sandwich hybridization assay (SHA) and quantitative real-time PCR (QPCR) using the globally-distributed ichthyotoxic raphidophyte *Heterosigma akashiwo* as a study organism. Both SHA and QPCR are cell homogenate approaches that use DNA probes to target sequences of interest. SHA detects ribosomal RNA (rRNA) from an unpurified and unamplified sample by hybridizing two DNA probes then quantifying rRNA colorimetrically whereas QPCR entails DNA purification then quantifying amplified DNA as fluorescence emission. Both approaches have been validated for *H. akashiwo*, but they have not been rigorously compared representing a gap in the ability to provide recommendations to researchers and managers for HAB activities. We describe laboratory and field studies examining how a wide range of population and environmental factors influence SHA and QPCR. For all samples, homogenates were split and processed using both approaches. Specifically, we compared standard curves of geographically distinct strains, assay ranges and limits of detection, and the consequences of preservation using Lugol's iodine solution on sample integrity over time. We also describe our monitoring and assessment validation efforts and how strain variability may translate to field observations. All findings to date showed that quantification using either SHA or QPCR exhibited comparable results.

P-63 ST-02

Monitoring *Alexandrium fundyense* using Fluorescent *in-situ* Hybridization and an Imaging Flow Cytometer (FlowCAM®) for early detection of toxic bloom events in Harpswell, Maine

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The ability to detect, identify and enumerate harmful algal species is a requirement for monitoring programs and early detection of harmful bloom events in coastal ecosystems. Monitoring programs utilize microscopes for identifying bloom species in a laboratory from field samples, which can be laborious and time consuming. FlowCAM is an imaging-flow-cytometer that combines the capabilities of a flow cytometer with a digital-imaging microscope and automates phytoplankton detection and enumeration. Here, we combine FlowCAM and fluorescent in-situ hybridization (FISH) analysis to target *Alexandrium fundyense* for two bloom seasons in Harpswell, Maine. A modified FISH method was used with FlowCAM to determine *Alexandrium fundyense* cell concentrations at critical management thresholds (50-500 cells/L) as an early warning from May through July, the time of year most critical to toxic shellfish events. Results from 2013 and 2014 demonstrate the ability of this technology to accurately detect *Alexandrium fundyense* cell concentrations using a high throughput, automated enumeration process while producing an archival collection of images for further analysis and comparison from year to year.

P-64

Multi-year glider observations of HAB triggers and events off the Southern California Coast

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Toxic blooms of *Pseudo-nitzschia* are a recurring problem in the central southern California Bight south of the Port of Los Angeles. Some the highest DA concentrations per cell for a natural population have been reported from this region. Multi-year (2012-2014) winter and spring season studies included autonomous glider deployments weekly pier data for phytoplankton, temperature and nutrients, and ocean color data. These data sets were combined to provide a multidimensional view of the development and evolution of these harmful algal blooms (HABs) as part of a NOAA-funded EcoHAB project.
This combined data sources were used to better understand the spring seasonal variation in phytoplankton blooms related to many factors including surface advection, upwelled nutrients and subsurface seeding of surface blooms. The gliders were equipped with a Sea-Bird conductivity-temperature-depth sensor and optical sensors including a three channel fluorometer for chlorophyll a, colored dissolved organic matter (CDOM) and phycoerythrin/ rhodamine and a 3 wavelength backscatter sensor. The results indicate significant interannual variation with local upwelling often being a trigger mechanism for HABs as subsurface, nutrient rich water and embedded subsurface phytoplankton populations are transported to the surface. But upwelling is only a portion of the processes contributing to the presence of HABs in the region. HAB populations can be advected into the region from the north. The initiation, intensity and duration of HAB events depend, in part, of the transport of nutrients and phytoplankton populations from the north (poleward).

P-65 ST-01

Real-time monitoring of harmful algae in the U.S. Pacific Northwest using the Environmental Sample Processor

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Shellfish harvesting closures due to contamination from biotoxins commonly occur in the coastal waters of the U.S. Pacific Northwest. Closures can occur with little warning or even after harvest, resulting in costly recalls of contaminated product. The Environmental Sample Processor (ESP) offers timely, quantitative, *in-situ* detection capabilities by filtering water samples and analyzing them onboard. Specific harmful algal and bacterial targets are detected in near-real time (~3 h) using DNA and RNA-based technologies. In the summer of 2012 and 2013, five ESP deployments totaling ~100 days provided advanced warning of HABs in the inland coastal waters of Puget Sound to support proactive fisheries management efforts. Deployment sites included a tribal shellfish and finfish hatchery and a commercial shellfish farm. The ESPs were configured to sample daily and detect *Heterosigma akashiwo*, *Alexandrium* spp., and *Pseudo-nitzschia* spp. along with bacterial targets and microbial source-tracking indicators at one location. ESP observations were confirmed by microscopy of manually collected validation samples as well as community composition profiling of archived material collected onboard the instrument using molecular bar coding methods. *H. akashiwo* was detected during both years and at low densities confirming the ESP's ability to detect HABs before they are able to contaminate/harm shellfish/finfish and threaten public health or economic interests. Data generated by the ESPs were rapidly distributed to end-users via online data visualization tools including the Pacific Northwest regional ocean observing system of the Integrated Ocean Observing System. This ability makes the ESP a useful tool for early warning of HABs.

P-66

Ribosomal DNA patterns and new chromosomal features in the dinoflagellate genus *Alexandrium*

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Dinoflagellates are a group of protists whose genome differs from that of other eukaryotes in terms of size (contain up to 250pg per haploid cell), base composition, chromosomal organization, and gene expression. But rDNA gene mapping of the active nucleolus in this unusual eukaryotic genome has not been carried out thus far. We used FISH in dinoflagellate species belonging to the genus *Alexandrium* (genome sizes ranging from 21 to 170 pg of DNA per haploid genome) to localize the sequences encoding the 18S, 5.8S, and 28S rRNA genes. Our results indicate that each dinoflagellate cell contains only one active nucleolus, with no hybridization signals outside it. However, the rDNA organization varies among species, from repetitive clusters forming discrete nuclear organizer regions (NORs) in some to specialized “ribosomal chromosomes” in others. These chromosomes, never reported before in other eukaryotes, are mainly formed by rDNA genes and appeared in the species with the highest DNA content. In addition, dinoflagellate chromosomes are first characterized by several eukaryotic features, such as structural differentiation (centromere-like constrictions), size differences (dot chromosomes), and SAT (satellited) chromosomes. NOR patterns prove to be useful in discriminating between cryptic species and life cycle stages in protists.

P-67

Does the pinnatoxin-producing dinoflagellate, *Vulcanodinium rugosum*, comprise a species complex?

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Pinnatoxins are members of the toxic cyclic imine group and were first characterized over two decades ago. However, the dinoflagellate producer of pinnatoxins was not known until 2011 when cultured from sediment samples in New Zealand. The causative species, *Vulcanodinium rugosum* Nézan & Chomérat, was described in 2011 from a bloom sample collected in a Mediterranean lagoon. Since then *V. rugosum* has been isolated from diverse geographic locations around the world including in Australia, Japan, China, Hawaii, Mexico, Qatar, and the United States. *V. rugosum* strains of various origins produce different forms of pinnatoxins and also differ from each other in partial large subunit rDNA and internal transcribed spacer regions suggesting the existence of a species complex. However, there is little morphological differentiation between isolates from genetically divergent populations, suggesting these populations may have recently diverged. This study sought to determine the status of the species complex by combining DNA sequence data, morphological characteristics, toxin profiles, and outcomes of mating crosses from strains originating from various geographical locations.

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Species diversity of the marine diatom genus *Pseudo-nitzschia* (Bacillariophyceae) along Chinese coastal waters

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Pseudo-nitzschia Peragallo is a widely distributed genus restricted to the marine plankton, and its species have attracted much attention after the first intoxication incident occurred in Canada in 1987. So far, more than 40 *Pseudo-nitzschia* taxa have been recorded. But in China, studies on *Pseudo-nitzschia* are still in their infancy. And until now, the identity of *Pseudo-nitzschia* species has caused confusion in China because most studies have been conducted under light microscopy and few of the previous studies have showed illustrations of the species. For the detailed study on species diverisy of *Pseudo-nitzschia* along Chinese coastal waters, the identifications based on the combination of morphology under transmission electron microscopy and molecular phylogenetic analyses inferred from ITS sequence are performed. From 66 established monoclone strains, a total of 19 taxa and varieties are confirmed, i.e., *P. americana*, *P. brasiliana*, *P. caciantha*, *P. calliantha*, *P. cuspidata*, *P. delicatissima*, *P. dolorosa*, *P. galaxiae*, *P. mannii*, *P. micropora*, *P. multiseries*, *P. mutistriata*, *P. plurisecta*, *P. pseudodelicatissima*, *P. pungens* var. *aveirensis*, *P. pungens* var. *pungens*, *P. sinica*, *P. subfraudulenta* and *P. subpacifica*. Five taxa are new records for China, two are new for Asia. We found a high diversity of *Pseudo-nitzschia* along Chinese coast and the species diversity must therefore be considered much higher than previously thought. Detailed morphometric data are obtained and some variations of morphological characters are discussed. This study may serve as a baseline study for further studies on *Pseudo-nitzschia* in China and add information to its global occurrence and taxonomy.

P-69

Small, potentially fish-killing dinoflagellates in the coastal Northern Baltic

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Studies are increasingly showing the importance of relatively small hetero- and mixotrophic dinoflagellates in coastal marine ecosystems, both as components of microbial food webs and as potential “harmful algae” implicated in fish kills. In the Baltic Sea, the basic occurrence and diversity of such small dinoflagellates is poorly known compared to larger phototrophic taxa. A study on the diversity of small hetero- and mixotrophic dinoflagellates in the Northern Baltic Sea was carried out in June-August 2013. Field samples collected from the vicinity of Hanko peninsula and Åland Islands were examined and 512 small (approximately 10-30 µm) dinoflagellate cells were isolated into clonal culture. Sequencing of the LSU- SSU- and ITS-rDNA revealed the presence of species belonging to Pfiesteriaceae as well as of the species *Karlodinium veneficum* in the Northern Baltic Sea. Morphological identity was corroborated by scanning electron microscopy, and genetic relatedness to previous geographic clades was investigated. *K. veneficum* and *Pfiesteria piscicida* have been associated with fish kills worldwide and are considered potentially harmful species. Tests for Karlotoxin were, however, negative for the Baltic isolates of *K. veneficum*. To our knowledge this is the first detailed description of *Cryptoperidiniopsis spp.*, *K. veneficum* and *P. piscicida* in the Baltic.

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Whole community profiling of harmful algae; sequencing 18S rRNA amplicons on an Ion torrent PGM

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Monitoring for harmful algal species is a costly and time consuming process. Cell counting using light microscopy is undertaken in many countries for a variety of species, i.e *Alexandrium, Pseudo-nitzschia, Dinophysis.* However, identifying some toxic species and strains either requires a high degree of taxonomic knowledge or is not possible. We investigated the suitability of using high throughput sequencing to rapidly assess the whole phytoplankton community composition, identifying harmful algal species and subsequently looking at their seasonal dynamics. Phytoplankton samples collected from Scapa Flow in the Orkney Islands throughout 2010 and 2011, were counted and identified using light microscopy, DNA extracted and a small fragment of the 18S rRNA gene (V9 region) amplified and sequenced using an Ion torrent PGM.
Dinoflagellates and diatoms dominated the sequence libraries in all months with the relative abundance of toxic genera such as *Alexandrium, Karenia, Prorocentrum* and *Pseudo-nitzschia* matching the count data well. We were also able to detect difficult to count genera such as *Chrysochromulina spp* and members of the Dictyochophyceae. The advantage of the Ion torrent over other sequencing platforms is that its 2 day workflow (from sample to data) and cheap cost means that monitoring programs could potentially incorporate this level of analysis into their protocols. This assay could be further developed to target multiple regions of rRNA and ITS to give better taxonomic resolution and also sequence longer fragments (<400bp) this study is one of the first to investigate the use of Ion torrent to monitor harmful algal populations.

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Morphology and molecular phylogeny of potentially undescribed marine benthic dinoflagellates isolated from Japan, Indonesia and the Philippines

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Benthic dinoflagellates comprised both toxic (or potentially toxic) and non-toxic species that contribute many important aspects in the marine environment. It has been suggested that in order to understand the diversity and distribution of these benthic dinoflagellates, clear description of their identities is needed. This study is aimed to confirm the taxonomy and phylogeny of several benthic dinoflagellate strains isolated from tropical and subtropical marine areas in Southeast Asia and Japan. Three strains of benthic and armored dinoflagellate were isolated from seagrass bed area in Ishigaki Island, Okinawa, Japan in July 2010 and they were characterized by light and scanning electron microscopy. The strains were described as having motile cell that is somewhat ovoid in shape with protruding apical pore complex (APC) and cingulum that is slightly displaced in mid-ventral view. The thecal plate tabulation of these strains was defined as: APC, x, 4`, 2a, 6``, 6c, 4s, 5```, 2. Subsequently, three additional strains that morphologically very similar to the Ishigaki strains were also isolated separately from Aka Island (Japan), Pari Island (Indonesia) and Masinloc Bay (Philippines). Despite the partial LSU rDNA D1D3 sequence of these strains showed >98% similarity and they were closely linked to *Peridiniopsis borgei* Lemmerman, however less than 90% base pairs were shared with this species or other morphologically similar species. Based on the morphological and molecular data, we suggested that these strains represent an undescribed species of marine benthic dinoflagellate.

P-72

First detection of a bloom forming *Peridiniopsis* species from a Sardinian reservoir (NW Mediterraean Sea)

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In the Mediterranean region, reservoirs represent the main sources of water supply for various human demands. The main issues in these ecosystems are linked to eutrophication and, consequently, to the development of Cyanobacteria blooms. However, freshwater red tides caused by dinoflagellates are becoming a new emergent problem for water treatments. In Sardinian reservoirs, dinoflagellate blooms have been detected since the seventies and were mainly caused by *Ceratium hirundinella*. Recently, other dinoflagellates have given rise to similar events. One of the most intense was recorded in Cedrino Lake in February 2012. The causative dinoflagellates were firstly recognized as *Peridinium* species. In this study, fixed samples collected during this event were analyzed in more detail with optical and scanning electron microscopy. Furthermore, surface sediment samples were taken and analyzed for the presence of resting cysts. The plate formula of the recovered cells was po, x, 4', 6'', 5C, 4S, 5''' and 2''''. Scattered pores ornamented the theca and numerous spines the hypotheca. Based on the tabulation features, this dinoflagellate was assigned to the *Peridiniopsis* genus, 'penardii' section. The cysts were slightly irregular in shape, and most of them maintained the external theca. The cyst content was grainy with yellowish to reddish accumulation bodies. Some inconsistencies (on cell and cyst morphology) found in the literature, have not permitted the certain determination of the species. Those difficulties fall in a larger scientific context because many of the known freshwater dinoflagellates have been described before the coming of electron microscopy and molecular methods.

P-73

Cyanobacteria diversity in four reservoirs in a semi-arid region of northeast Brazil

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Cyanobacteria diversity in four reservoirs from a semi-arid region of northeast Brazil was analyzed by morphological criteria and denaturing gradient gel electrophoresis (DGGE), followed by DNA sequencing. The DGGE profile showed a complex pattern and higher number of OTUs with high diversity when compared with diversity based on morphotypes. The low values of similarity coefficient (<40%) indicated variation of the population's genetic diversity as well as a heterogeneous spatial distribution of community along the studied reservoirs. Cyanobacteria diversity based on 16S rDNA sequences were higher than that based on morphotypes within and between reservoirs. A total of 54 phylotypes were obtained, however 43 showed no corresponding with morphotypes. The dominant cyanobacteria species from the excised DGGE bands were related to *Planktothrix* (32%), *Mycrocystis* (20%). Other phylogenetic groups identified included species associated to *Calothrix, Tolypothrix, Cylindrospermum* and *Chroococcidiopsis* (20%). The Detrended Correspondence Analysis (DCA) showed that the diversity and the spatial distribution of the cyanobacteria (morphotypes and phylotypes) were related to high nutrient concentrations, turbidity, total organic matter, biochemical oxygen demand and depth. Support: CNPq.

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New *Ostreopsis* species recorded along Cyprus coasts: toxic effect and preliminary characterization of chemical-molecular aspects

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The genus *Ostreopsis* E.J. Schmidt includes harmful benthic species that have been reported worldwide in both tropical and temperate regions. To date, genetic studies stated that Mediterranean Sea hosts two genotypes corresponding to the morphotypes of *O.* cf. *ovata* and *O.* cf. *siamensis*; moreover, a new genotype of *Ostreopsis* sp. was found along Greece and Cyprus coasts. Nowadays, reliable data on harmful algal bloom and cases of poisoning associated in the eastern Mediterranean basin are still scarce. This study describes, for the first time, toxic effects and chemical-molecular aspects of the Cypriot genotype of *Ostreopsis* sp. (C1036-2013). Ecotoxicological bioassays were performed exposing *A. salina* nauplii to the following treatments of *Ostreopsis* sp.: untreated culture, filtered and resuspended cells in fresh medium, resuspended and sonicated cells in fresh medium, growth medium devoid of algal cells by 6 µm (mucilage remains in the treatment) and 0.22 µm mesh size filtration. Our results show higher toxic effects (mortality and immobility) with the untreated *Ostreopsis* sp. culture ($LC_{50\text{-}48h}$ = 45 cells/ml, $EC_{50\text{-}48h}$ = 7 cells/ml). As regard lethal effects, the Cypriot strain seems to be less toxic than the most widespread species *O.* cf. *ovata* ($LC_{50\text{-}48h}$ < 4 cells/ml), though further studies are needed to better understand the toxicity of this new genotype. Additionally, preliminary data on its toxic profile reports presence of new palytoxin-like compounds and the lack of palytoxin and all ovatoxins so far known, confirming the possibility of being considered a different species.

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New species of the genus *Prymnesium* from the Bay of Biscay (Spain) and Gold Coast (Australia)

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Two marine species of the potentially toxic genus *Prymnesium* are described by means of light and electron microscopy and analysis of the small (SSU) and large (LSU) subunit of ribosomal DNA. One of the species was brought into culture from surface samples collected on the seaward part of the Nervión river estuary (Bay of Biscay, Spain), the other one was collected from the same site and also from the eastern coast of Australia (Gold Coast). Both were isolated into clonal cultures by micropippeting single cells. The cells are similar in shape and size to other members of the genus *Prymnesium*, but the ornamentation of the scales covering the cells and phylogenetic analyses based on LSU and SSU rDNA sequences show significant differences indicating that they are new species.

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Morphological, genetic, and toxicological characterization of *Pyrodinium bahamense* from the Gulf of California

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A morphometric analysis compared five *Pyrodinium bahamense* populations in the Gulf of California (related to var. *bahamense*) with one from the Gulf of Tehuantepec (related to var. *compressum*). Genetic and toxicological characterization of strains isolated from one site in the Gulf of California was also conducted. Specimens (n = 100) of each population were measured and photographed, toxicity was analysed by ELISA immunoassay method, and genetic characterization was done by amplification, sequencing, and alignment analyses of sequences corresponding to the LSU D1-D2 domains (667 bp), using the universal Euk primers and SSU-ITS-LSU (400 bp). Morphometric data of vegetative cells showed wide dispersion and overlap, demonstrating an important morphologic intra- and inter-population variability. When we applied a non-parametric statistical method, the populations could not be distinguished from each other ($\alpha = 0.05$). Saxitoxin concentration ranges from 1–48 pg STX mL^{-1}. These results suggest that populations from the Gulf of California can produce paralytic shellfish toxins. The sequence alignment analyses of the LSU D1-D2 domains resulted on 99% identity with the three entries of *P. bahamense* var. *compressum* (GenBank accession numbers AY566192.1, AY566191.1, and AY154959.1). Morphological and toxicological characteristics that are used to separate *P. bahamense* into two varieties are not suitable. Thus, it is necessary to carry out studies to define the effect of environmental variables in the morphology and life cycle of *P. bahamense* and to intensify research on genetic and toxicological variability of the species at regional and global scales.

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Cellular responses associated with ROS production and cell fate decision in response to short-term iron limitation in the diatom *Thalassiosira pseudonana*

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Diatoms are believed to be responsible for around 25% of global annual primary production and often form massive blooms. The discovery of the distinct strategies and cellular responses at the protein level that these organisms use to cope with the rapid fluctuations in iron availability may facilitate a better understanding of their anti-stress capability and population control during blooms. In this study, using a combination of biochemical markers, *in vivo* cell fluorescence staining, and whole-cell iTRAQ-based proteomics analysis, we explored the cellular responses associated with ROS production and cell fate decision exhibited by the diatom *Thalassiosira pseudonana* in response to short-term Fe limitation. Fe limitation (four days) caused a significant decrease in PS II photosynthetic efficiency, damage to the photosynthetic electron transport chain in PS I, and blockage of the respiratory chain in complexes III and IV, which resulted in excess ROS accumulation. The increase in ROS then triggered programmed cell death (PCD) in some of the Fe-limited cells through a series of proteins involved in the delicate balance between pro-survival and pro-PCD factors associated with the cell fate decision. The results provide molecular-level insights into the major strategies that are employed by *T. pseudonana* in response to short-term Fe-limitation stress: the reduction of cell population through PCD to reduce competition for the available Fe, the enhancement of reallocation of intracellular nitrogen and Fe into key cellular components to ensure the most basic survival, and the increase in expression of antioxidant and anti-PCD proteins to ensure survival of the population.

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Discovery of non-coding small RNAs in *Amphidinium carterae* differentially expressed over a diel cycle

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Although proteins involved in photosynthesis and replication have been shown in dinoflagellates to have differential expression patterns over a diel cycle, mRNA transcripts for these genes do not change in concert. This indicates that protein expression is controlled post-transcriptionally, possibly by selective recruitment/degradation or by altered translation kinetics. We extracted RNA at thirteen timepoints from 6 hours before the light/dark shift to 6 hours after from an actively dividing culture of *Amphidinium carterae* (CCMP1314). Other than a global reduction in expression at the light/dark transition, no differential expression of mRNAs for proteins of metabolism, photosynthesis, translation initiation, or ribosomal RNAs was observed. However, a doubling in total extractable RNA at +4 hours was observed, hence a library was constructed of size selected (<200 bp) RNAs and Illumina sequencing was performed resulting in 2770 unique sequences. Along with nuclear and organellar ribosomal RNAs, tRNAs, a putative spliced leader RNA, and organellar gene fragments; many RNAs were found that did not contain recognizable open reading frames. These RNAs were often polyadenylated, with expression levels approaching 1.38 million mapped reads per thousand bases per million reads (RPKM) compared to 600 RPKM for ribosomal genes. Of these, 94 matched annotated sequences in miRBase and 1215 were differentially expressed at a single timepoint with low or no expression at all others. These non-coding RNAs could be responsible for translation repression or activation of specific mRNAs.

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Heterosigma and *Olisthodiscus* – New insights

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Heterosigma akashiwo is the smallest known raphidophyte and it is renowned for its ability to form dense, fish-killing algal blooms. In large parts of the world *H. akashiwo* is a permanent resident of the algal community, and blooms have caused extensive financial losses for the aquaculture industry. Much work have been done to understand this species over the last two decades, but perhaps because of its small size and fragile nature, few morphological studies have been conducted. We collected available *Heterosigma akashiwo* strains from Europe and North America and conducted extensive genetic and morphological studies. 18S and the D1-D2 region of 28S rDNA were sequenced and compared with all other available raphidophyte sequences. A new genotype was revealed, probably representing a sister species to *H. akashiwo.* Further, a new species of raphidophyte were discovered in Danish water, a close relative of *Olisthodiscus luteus.* The two new genotypes along with an American and a European *H. akashiwo* have been extensively studied in SEM and TEM.

P-80

Genetic aspects for the elucidation of volatile organic compounds production by cyanobacteria

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Cyanobacteria have a remarkable ability to produce many bioactive metabolites including volatile organic compounds (VOCs). Some of the VOCs such as β-cyclocitral and β-ionone showed lytic activity and may be involved in the regulation of the occurrence of cyanobacteria. The genetic aspects for investigation of β-cyclocitral, β-ionone and alcohols production mechanism by several species of cyanobacteria including three strains of *Microcystis aeruginosa*, *Anabaena lemmermannii, Pseudanabaena* sp., *Synechocystis* sp. and *Plantkothrix agardhii* have been taking into consideration. The β-cyclocitral and β-ionone appear to be produced by oxidative cleavage of carotenoids catalysed by carotenoid cleavage dioxygenase (CCD). The biosynthetic mechanism of alcohols is independent and is catalysed by 2-keto-acid decarboxylase (KDC) and alcohol dehydrogenase (ADH). The verification of specific enzymes-encoding sequences in genomic DNA of studied cyanobacterial species was made. The results of molecular analysis confirmed the presence of two CCD-encoding sequences and one KDC-encoding sequence in genomic DNA of each strain of *Microcystis aeruginosa* that were revealed high homology (above 95%) in contrast to the other studied cyanobacterial species. These findings are greatly contributed to the explanation of the varieties in production behaviour of VOCs by different species of cyanobacteria.

P-81

Environment to the cell: a systems study on the physiological ecology, toxin and proteome profiles of P*yrodinium bahamense* var c*ompressum* isolated from Sorsogon Bay, Philippines

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In 2010, rise in temperatures because of El Niño phenomenon has caused proliferation of Harmful Algal Blooms in four Bay areas in the Philippines including Sorsogon Bay. One of the major HAB-causing species identified in the said bloom is *Pyrodinium bahamense* var *compressum*. In the laboratory, cultures of the *Pyrodinium bahamense* var *compressum* isolated from Sorsogon Bay were studied based on its ecophysiological response on differing salinities and light intensities. Results have shown that *P. bahamense* var *compressum* tolerated a wide range of salinities except at 15 psu. It was shown to grow at low to high light intensities (80 to > 200 $\mu Em^{-2}s^{-1}$). At high light intensities, significant toxicity increase (>15-fold) was observed between mid- to late exponential phase as observed in a 21 day toxin profiling. Proteome profiles generated from two-dimensional gel electrophoresis revealed upregulated or optically dense protein spots at the mid- to low molecular weight region. Further, MALDI-TOF and multi-layered bioinformatics were performed for protein identification. Results revealed difference in regulation of chaperonins, oxidoreductases, etc. With the onset of El Niño this 2014 and its possible occurrence on the succeeding years, this study provides a systems insights from the environmental to the molecular aspect of growth and toxin production of *P. bahamense* var *compressum*- Sorsogon Bay strain in better understanding its bloom dynamics.

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Identification of two marine woloszynskioid dinoflagellates (Suessiaceae) from Vietnam based on morphology and molecular phylogeny

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Two marine planktonic dinoflagellates, superficially resembling *Gymnodinium* spp., were collected from the southern coast of Vietnam and observed by light and scanning electron microscopy (SEM). Their phylogenetic positions were inferred from partial LSU rDNA (D1–D3) sequences. One species was identified as *Pelagodinium beii* in the family Suessiaceae. Morphological characters of Vietnamese *P. beii* basically coincided with the original description, but had small differences in the number of amphiesmal vesicles (AVs); five AVs in the first epiconal series and three AV series in the cingulum is different from the previous report of *P. beii* from the Caribbean Sea. Vietnamese *P. beii* was branched at the basal position of the *Pelagodinium* clade in phylogenetic tree. Another species was an undescribed species in the Suessiaceae. Cells of the undescribed species were ovoid, having yellowish chloroplasts and an eyespot at the sulcal region. The apical furrow was present. SEM also revealed characteristic features of the Vietnamese woloszynskioid, the few numbers of AVs less than 30 in total, and the peduncle emerged from ventral side of the epicone. Among the previously described species in the Suessiaceae, the smallest AV number is more than 50 (*Symbiodinium* spp.). The peduncle is usually situated between the two flagella in many other dinoflagellates, and the peduncle located in the epicone has been reported only from *Moestrupia oblonga* and *Prosoaulax lacustris*, both are not related to the Suessiaceae. Phylogenetic position of the undescribed species was the base of the Suessiaceae clade.

P-83

Understanding strategy of nitrate and urea assimilation in a Chinese strain of *Aureococcus anophagefferens* through RNA-seq analysis

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Aureococcus anophagefferens is a harmful alga that dominates plankton communities during brown tides in North America, Africa, and Asia. Here, RNA-seq technology was used to profile the transcriptome of *A. anophagefferens* that was grown on urea, nitrate, and a mixture of urea and nitrate, and that was under N-replete, limited and recovery conditions to understand the molecular mechanisms that underlie nitrate and urea utilization. The number of differentially expressed genes between urea-grown and mixture N-grown cells were much less than those between urea-grown and nitrate-grown cells. Compared with nitrate-grown cells, mixture N-grown cells contained much lower levels of transcripts encoding proteins that are involved in nitrate transport and assimilation. Together with profiles of nutrient changes in media, these results suggest that *A. anophagefferens* primarily feeds on urea instead of nitrate when urea and nitrate co-exist. Furthermore, we noted that transcripts upregulated by nitrate and N-limitation included those encoding proteins involved in amino acid, nucleotide and aminosugar transport, degradation of amides and cyanates, and nitrate assimilation pathway. The data suggest that *A. anophagefferens* possesses an ability to utilize a variety of dissolved organic nitrogen. Moreover, transcripts for synthesis of proteins, glutamate-derived amino acids, spermines and sterols were upregulated by urea. Transcripts encoding key enzymes that are involved in the ornithine-urea and TCA cycles were differentially regulated by urea and nitrogen concentration, which suggests that the OUC may be linked to the TCA cycle and involved in reallocation of intracellular carbon and nitrogen.

P-84

Transcriptional responses of *Hsp70* gene to temperature stresses in the cosmopolitan HAB-forming dinoflagellate *Akashiwo sanguinea*

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Heat shock proteins (Hsp70) are a class of molecular chaperones well-known for their participation in defending against heat stresses in many plants including macro- and microalgae. The unarmoured dinoflagellate *Akashiwo sanguinea* is a cosmopolitan species frequently causing harmful algal blooms in marine estuaries and coasts from temperate to tropical waters. While the wide temperature tolerance has been well documented as one of its typical adaptive strategies, much is unknown, however, about the functional genes involved in this ecological process. We investigated the possible role of *Hsp70* gene in the temperature adaptation strategy of *A. sanguinea*. Based on homologous cloning and the rapid amplification of cDNA ends (RACE), the full-length cDNA sequence (2171 bp) of *Hsp70* gene from *A. sanguinea* (AsHsp70) was obtained (Accession No. KJ755185), with an open reading frame of 1950 bp. The deduced 649 amino acids (aa) possessed an ATPase domain of 399 aa, a substrate peptide binding domain of 124 aa, and a C-terminus domain of 126 aa. The highly conserved motifs of Hsp70 family were identified in AsHsp70, suggesting the conservation of Hsp70 function. Real-time quantitative PCR revealed that the *AsHsp70* expression (20°C-maintained) was dramatically up-regulated by both low (15°C, 10°C, 5°C) and high (25°C, 30°C) temperature shocks. Rapid and significant increase of *AsHsp70* expression could be detected in 10 min of exposure to 10°C in the time-course experiment. Comparison between the results of stepwise increase (or decrease) in temperature (± 5°C each step) and that of one-step shocks (± 10°C) observed significantly higher *AsHsp70* transcripts in the more drastic stresses. These results together strongly suggest that *A. sanguinea* is more tolerable to gradual temperature stress and that *AsHsp70* is involved in the adaptation of *A. sanguinea* to the varying temperature.

P-85

Comparison of three *Pseudo-nitzschia* species through transcriptome sequencing

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The genus *Pseudo-nitzschia* comprises about 37 species of pennate planktonic diatoms recorded in both coastal and open oceanic waters. Some of these species are known to produce the neurotoxin domoic acid (DA), the molecule responsible for Amnesic Shellfish Poisoning. Taking advantage of sequencing projects sponsored by the Gordon and Betty Moore Foundation (http://www.moore.org/marine-micro.aspx) and the JGI institute (http://www.jgi.doe.gov/), we generated transcriptomes for three *Pseudo-nitzschia* species: *P. arenysensis*, *P. delicatissima* and *P. multistriata*, the last one able to produce DA.
The comparison of transcriptomes revealed a high similarity in proteome content and pathways utilization between *P. arenysensis* and *P. delicatissima*, while in *P. multistriata* different trends could be observed in a number of cases, for instance in the pentose phosphate and calvin cycle pathways utilization as well as in fatty acid biosynthesis and oxidation. The proteome comparisons were expanded, including datasets from *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*, with the aim to identify unique features. We defined the orthologous (inter-species homologous sequences) and paralogous (intra-species homologous sequences) transcripts among the five diatoms, using the OrthoMCL program. Overall, we could detect species-specific or genus-specific sequences, for example in the transcription factors family types and content. The *in silico* analyses also provided interesting candidate genes for DA production in *P. multistriata*, experimental validations are ongoing to confirm their involvement in DA biosynthesis.

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Physiological and transcriptomic responses of the harmful dinoflagellate *Cochlodinium polykrikoides* exposed to algicide agents

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The dinoflagellate *Cochlodinium polykrikoides* can form harmful algal blooms (HABs) that cause lots of fish death followed by huge economic loss. Here, we assessed physiological and transcriptomic responses of *C. polykrikoides* exposed to algicides NaOCl and copper sulfate ($CuSO_4$). The test organism showed a dose-dependent decrease in growth rate against both algicides; 72-h median effective concentration (EC_{50}) was 0.584 $mg \cdot L^{-1}$ and 0.633 $mg \cdot L^{-1}$ for NaOCl and $CuSO_4$, respectively. The decrease in pigment levels and intensity of chlorophyll autofluorescence (CAF) showed that NaOCl and $CuSO_4$ might affect the photosynthetic processes of the algicide exposed cells. In addition, we analyzed the dinoflagellate genomic response in terms of gene transcriptional regulation. HiSeq RNA-sequencing generated 173 million reads, which were further assembled to 191,212 contigs. 43.28%, 33.9%, and 15.6% of contigs annotation of were achieved by homologous searches with NCBI NR database, GO database, and KEGG database, respectively. KEGG pathway analysis revealed that translation, signal transduction, and carbohydrate metabolism were the top three of the highest up-regulated pathways; on the other hand, translation, signal transduction, and energy metabolism were the top three down-regulated pathways. Particularly, we found that the photosynthesis and oxidative phosphorylation were the highest up- and down- regulated in the terms of metabolism process pathway. Additional experiment showed that the photosynthesis was particularly inhibited, and reactive oxygen species (ROS) production was increased by $CuSO_4$. These results suggest that photosynthetic pathway of *C. polykrikoides* may be severely affected by algicide exposures, contributing to cell death of the bloom-forming dinoflagelalte.

P-87

Morphology of *Gambierdiscus scabrosus* sp. nov.: a new epiphytic toxic dinoflagellate from coastal areas of Japan

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Here we described a new epiphytic dinoflagellate, *Gambierdiscus scabrosus* sp. nov. which was reported as toxic *Gambierdiscus* sp. type 1 from coastal areas of Japan by Nishimura et al. (2013). Cells of *G. scabrosus* are 63.2 ± 5.7 µm in depth, 58.2 ± 5.7µm in width and 37.3 ± 3.5µm in length. The plate formula of *G. scabrosus* is Po, 4′, 0a, 6″, 6c, ?s, 5‴, 0p and 2⁗. Morphologically, *G. scabrosus* resembles *G. belizeanus* in its anterioposteriorly compressed cell shape, narrow 2⁗ plate and areolated surface. Despite this similarity, the cells of *G. scabrosus* can be distinguished by the presence of the asymmetric shaped 3″ plate and the rectangular shaped 2′ plate.

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Dynamics of *Gambierdiscus* spp. around Tosa Bay, Japan between 2007 and 2013

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Cell densities of *Gambierdiscus* spp. in coastal areas of Tosa Bay, Japan were assessed monthly under LM observation between 2007 and 2013. The cell densities in the area ranged from 0 to 232.2 cells/ g fw algae. Mean cell densities assessed in spring, summer, autumn and winter were 0.1±0.4, 0.9±2.6, 4.0±20.6 and 0.4±1.4 cells/ g fw algae, respectively. The cell densities in summer and autumn were not significantly different ($p > 0.05$), whereas those in summer and autumn were significantly different from those in spring and winter ($p < 0.01$). A significant positive correlation was observed between the cell densities and sea surface temperatures (SST) ($r_s = 0.21$, n= 591, $p < 0.001$), while a significant negative correlation was observed between the cell densities and salinities ($r_s = -0.18$, n= 579, $p < 0.001$). As a consequence, these results suggest that cell densities of *Gambierdiscus* spp. increase in summer and autumn when SST is high and salinity is low.

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Characterization of *Gambierdiscus* and *Coolia* (Dinophyceae) isolates from Thailand based on morphology, phylogeny and toxicity

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The genus *Gambierdiscus* produces toxins that bioaccumulate in tropical and sub-tropical fish causing ciguatera fish poisoning (CFP). Other co-occurring genera such as *Coolia* have also been implicated in causing CFP. Little is known about the diversity and toxicity of the two genera *Gambierdiscus* and *Coolia* along the Thai coasts. In this study, we characterized *Gambierdiscus* and *Coolia* species collected for the Gulf of Thailand and the Andaman Sea based on observation under light microscopy (LM) and scanning electron microcopy (SEM) and phylogenetic analyses using the LSU rDNA sequences. The results of morphological and phylogenetical (LSU rDNA D8/D10) analyses revealed that strains of *Gambierdiscus* from Thailand enabled to be identified as *G. caribaeus*. In this study, some, but not all of the *Coolia* spp. strains from Thailand could be morphologically identified as *C. malayensis.* The LSU rDNA D1/D2 phylogeny confirmed the identity of *C. malayensis* isolates and placed the remaining in the *C. tropicalis* clade. The tested strain of Thai *G. caribaeus* revealed high toxicity to mice, whereas any toxicity associated with *C. malayensis* strain tested was below the limit of detection.

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Growth of the epiphytic dinoflagellate *Gambierdiscus caribaeus* under different temperature and nutrient concentration

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Recently, the occurrences of the subtropical several epiphytic dinoflagellates species were firstly reported from Jeju Island, the southern-most island of Korea (Jeong et al. 2013). Growth rates of an epiphytic dinoflagellate *Gambierdiscus caribaeus*, one of the laboratory strain established, were estimated through experimental cultivations. Under 30 psu, 60 μE m^{-2} s^{-1} condition experimental *G. caribaeus* cultures were tested for the effect of different nutrient concentrations (control, f/2, f/5, f/10 and f/20) on their growth for 3 months. The tests were carried out at two different temperatures i.e., 20°C and 25°C. The moderate nutrient concentration, f/5, supported the highest growth regardless of the test temperatures. The maximum density of *G. caribaeus* occurred on day 20 and days 50-80 under 25°C and 20°C, respectively. The experimental strain might be adapted to warmer season and the coastal waters of Jeju Island.

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The effects of temperature and salinity on the growth of the benthic dinoflagellate *Ostreopsis* spp. from Thailand.

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The benthic dinoflagellate *Ostreopsis* spp. are producers of palytoxin and its analogues. Although *Ostreopsis* spp. has been reported in the Thai coastal regions, little is known about their growth characteristics. Thus, the strains of *O.* cf. *ovata* Thailand subclade, *O.* cf. *ovata* South China Sea (SCS) subclade, *Ostreopsis* sp. 6 and *Ostreopsis* sp. 7 were used to clarify the effects of temperature and salinity on their growth. Four types of media (IMK, IMK/.2 f/2 and PES) were assessed. The f/2 medium gave the highest cell yields for all strains tested. The semi-optimal temperature and salinity conditions which gave >80 % of maximum growth rates of strains tested were 22.7–27.4˚C and 28.7–35 for *O.* cf. *ovata* Thailand subclade, 27.9–30.8˚C and 23.8–30.8 for *O.* cf. *ovata* South China Sea subclade, 23.5–26.4˚C and 29.8–36 for *Ostreopsis* sp. 6 and 23–27.2˚C and 28–36 for *Ostreopsis* sp. 7. Two-way ANOVA indicated that both temperature and salinity have a mutual influence (p<0.001) on the growth of *Ostreopsis* from Thailand. Our results demonstrate that the semi-optimal temperature-salinity conditions differ among Thai *Ostreopsis* species/phylotypes. Considering these results, temperature-salinity interaction may play an importance role in bloom dynamics and the distribution of the *Ostreopsis* species/phylotypes in Thai coastal water.

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First record of *Coolia monotis* Meunier along Alexandria coast- Egypt

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The distribution and abundance of epiphytic and planktonic *Coolia monotis* Meunier along Alexandria coast were studied through five annual cycles; from summer 2005 to summer 2010 at four sites: Abu Qir Beach, Stanly, Eastern Harbour (EH) and Mex Beach. The morphology of *C. monotis* was characterized by noticeable small size specimens in the EH. The D. V. diameter not exceeding 20 µm and width was between 10 - 15 µm. On the other hand, the epiphytic specimens were of a a normal cell size. The species was most abundant as planktonic form in the EH, reaching 15.2 x 10^3 cell l^{-1} during summer 2010. The epiphytic forms showed lower density, with a maximum of 454 cell g^{-1} fresh weight during autumn 2005, 2006 and summer from 2007 to 2010. The fluctuations of cell abundance of *C. monotis* showed a significant negative correlation with salinity and a weak positive correlation with temperature. This study represents the first report of *C. monotis* Meunier in the Egyptian Mediterranean waters.

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Analyses of different strains of *Prorocentrum lima* (Dinophyceae) by morphology, phylogeny and physiology

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Benthic microalgae are important components of the marine environment and widely studied throughout the world. In this study, we provide morphological, phylogenetic and photophysiological details of several strains of *Prorocentrum lima,* a cosmopolitan dinoflagellate, collected from different places across the Atlantic coast of the Iberian Peninsula, and compare it with the strain Dn141EHU collected from the Mediterranean Sea, which matched the original description of *Prorocentrum arenarium*. Although *P. arenarium* is widely accepted to be a synonym of *P. lima*, they show consistent morphological differences. For this study, cells were obtained by shaking macroalgae collected from rocky-shore areas bordering accessible beaches. Isolated strains were analysed for morphological characterization using LM and SEM. For phylogenetic characterization, the LSU and ITS1-5.8S-ITS2 regions of the rDNA were used as markers. The photophysiology was analyzed by means of fluorescence parameters by exposing the algae to different light intensities. Although, the analysed strains corresponded to a great extent to the original morphological characterization of each species, the phylogenetic analyses confirmed *P. arenarium* to be a synonym of *P. lima*. Despite the large differences observed between the two species in terms of morphological features, the phylogenetic and physiological analyses allow us to presume that these differences are intra-specific rather than inter-specific. Nevertheless, due to the huge morphological variation found in this study, it is suggested that new and more specific genes should be taken into account.

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Southernmost record of the invasive nuisance alga *Didymosphenia geminata* in Tierra del Fuego Island, Chile

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Didymosphenia geminata (Didymo) is a very aggressive invasive diatom, capable of producing large algal blooms, which cover the benthic substrate of freshwater environments, as a result of excessive extracellular stalk production. These algal mats, cause severe physical, chemical and biological changes in the rivers where they form these extensive mats. The first positive identification of Didymo in Chile was reported in 2010 in the Futaleufú and Espolón rivers, about 1700 km north of the Magellan region. Here we reported the first record of Didymo in Magellan region, specifically on Tierra del Fuego Island, Chile (53°53'35.21"S, 68°52'55.42"W). The study area comprised 73 freshwater sources within the Magellan region (53-56°S, which included four provinces: Ultima Esperanza, Magallanes, Tierra del Fuego and Antártica Chilena), which were classified according their anthropic use: fishing, kayaking, trekking, etc. The results obtained showed the presence of Didymo in two rivers of Tierra del Fuego Island Province: Grande and Blanco, covering an area of approximately 58 km of river affected continuously with positive samples of the invasive microalgae. The survey of rivers and lakes, established that most of the rivers in the Magellan region, constitute an ideal environment for the settlement of Didymo, so the eventual spread of the microalgae is imminent, if the prevention and biosecurity measures are not considered. We also discuss some concerns about the policy and management of Didymo and future research. This is the southernmost record of Didymo and the only record from Chile.

P-95

Environmental factors associated with *Didymosphenia geminata* blooms in Tierra del Fuego Island, southern Chile

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Didymosphenia geminata (Didymo) is a benthic diatom that was recorded for first time in Tierra del Fuego Island, southern Chile, at early 2013, in two connected freshwaters sources, Grande and Blanco rivers (53°53'34.94"S-68°52'54.75"W and 53°56'8.63"S-68°59'19.07"W, respectively). Shortly after the discovery of the nuisance alga, up to 58 km of benthic substrate were covered with a dense biofilm. Here we evaluate chemical (nitrate, phosphate, silicate and pH) and physical variables (temperature and conductivity). All parameters were measured under a monitoring program that was carried out during several months to associate the environmental variables with the presence of Didymo in these two rivers. The result showed an homogeneous behavior for pH, with values ranging from 7.4 to 7.8, temperature and conductivity with higher oscillation (5.3-17°C and 0.06-1.75 µS cm^{-1}) due mainly the seasonality of the sampling period; similar and very low total phosphate values (0.016-0.018 µM L^{-1}) and nitrate and silicates (ranging both between 0.018-0.067 µM L^{-1}) also with low concentrations. This is consistent with the available information suggesting that Didymo blooms occur much more frequently in rivers with oligotrophic waters. Future research is necessary to evaluate the importance of others variables such as iron and dissolved phosphorus on the triggering and settlement of Didymo blooms in this part of the world. It is also necessary to understand the hydrographic processes that play a key role in the spread of the nuisance microalgae on these oligotrophic freshwater environments in southern Chile.

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Didymosphenia geminata impacts on organisms vary related to differing effects of niche and neutral processes based on organism size.

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Didymosphenia geminata (Didymo) is an invasive freshwater diatom, whose effects on ecosystems and rapid spread in temperate regions is of international concern. *D. geminata* has been shown to alter habitats and primary, with subsequent effects throughout aquatic ecosystems. Assessing the impacts of an invasive, requires understanding of assemblage and diversity changes across broad spatial scales, whilst disentangling the effects of competing niche and neutral processes. We surveyed 55 sites across a gradient of *D. geminate* biomass within the South Island of New Zealand to determine the effects of *D. geminata* on algal and invertebrate ß diversity and assemblages. Increasing *D. geminata* biomass significantly reduced ß diversity (as community turnover) in algal (adonis P<0.001) and in invertebrate assemblages (adonis P<0.05). With increasing *D. geminata* biomass greater community homogeneity was observed in both algal (betadisper P<0.005) and invertebrate (betadisper P<0.05) assemblages. While *D. geminata* biomass strongly influenced algal composition, abiotic niche controls and space became increasingly important in structuring benthic invertebrate assemblages. This increased ecological determinism with a weakened effect of *D. geminata* on invertebrates, outlines the fundamental differences that exist between organisms and the effective scale of niche and neutral processes based on organism size. The scale of differences, and mechanisms causing these changes, further outline *D. geminata* may also be considered an ecosystem engineer.

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Environmental parameters promoting *Phormidium* blooms in New Zealand rivers

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Toxic benthic cyanobacterial blooms of *Phormidium* are becoming increasingly prevalent in New Zealand's rivers. There have been approximately 100 dog deaths in the last five years and human health warnings have been issued at multiple rivers. Despite the health risks, the development of effective management strategies is hampered by a limited understanding of what makes rivers vulnerable to blooms. The first aim of this study was to determine when, where and why *Phormidium* blooms in selected Canterbury rivers by comparing environmental variables across 10 lowland rivers. *Phormidium* was present in all rivers, however, proliferated only in five. The frequency and intensity of 'flushing flows' was found to be most important variable in determining the presence of blooms. However, the interaction between flow and substrate stability prevents the use of a single flushing flow value across all rivers. The majority of blooms were recorded at sites with moderate to high dissolved inorganic nitrogen (>0.10 mg/L). *Phormidium* proliferated when dissolved reactive phosphorus (DRP) concentrations were low (<0.010 mg/L). The second aim was to investigate alternative phosphorus sources. Preliminary results suggests that the ability of *Phormidium* to proliferate at low DRP concentration may be due to the development of zones within the mat matrix with high pH (>10) during the day and low dissolved oxygen (<1 mg/L) at night. These conditions may enhance availability of sediment-bound phosphorus trapped within mats. Planned future research will focus on this alternate phosphorus source, together with the possibility that other water quality attributes may influence *Phormidium* growth.

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Investigation of volatile organic compounds production behaviour of cyanobacteria

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Cyanobacteria are able to synthesize and release into the water a wide range of bioactive metabolites of various chemical structures. Some of them such as volatile organic compounds (VOCs) showed lytic activity, but their function in natural environment remains unknown. The conducted studies were focused on elucidation and comparison of the relationship between the growth and the VOCs production behaviour of several cyanobacterial species including *Microcystis aeruginosa, Planktothrix agardhii, Anabaena lemmermannii, Pseudanabaena* sp., and *Synechocystis* sp. The cyanobacteria were cultivated in laboratory conditions and sampled in regular time interval for the quantitative determination of the VOCs production (e.g. β-cyclocitral, β-ionone, and alcohols) using GC/MS. The simultaneous analyses of photosynthetical pigment concentration, number of cells and pH were measured. Amongst the detected alcohols the 1-butanol was found to be produced by cyanobacteria in the last stage of culturing. All studied species were producing β-ionone within whole period of culturing and its concentration was comparable with exception for *Synechocystis* sp. However, β-cyclocitral was synthesized during all growth phases only by *Microcystis aeruginosa* and its concentration was the highest in comparison to the other tested species. Based on the observations that the highest amounts of β-cyclocitral and β-ionone were detected together with the colour change of cyanobacterial cultures and decreasing of cells number, it can be assumed that these compounds may be involved in the regulation of the occurrence of cyanobacteria.

P-99

Effects of light and nutrients on the growth of *Microcystis ichthyoblabe* and *M. flos-aquae* isolated from the tropics

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The effects of light and nutrients on the growth of three hepatotoxic strains of *Microcystis* isolated from tropical reservoirs in Singapore (103°50'E, 1°20'N) were investigated in separate laboratory experiments. Two strains of toxic *M. ichthyoblabe* were isolated from two different reservoirs (Lower Peirce Reservoir and Tengeh Reservoir) and one strain of toxic *M. flos-aquae* was isolated from Lower Peirce Reservoir. Higher maximum growth rates for all three strains were observed at low (30 $\mu molm^2s^{-1}$) to medium light (55 $\mu molm^2s^{-1}$) intensities compared to high light (120 $\mu molm^2s^{-1}$) intensity, while very little growth was observed at very low light (10 $\mu molm^2s^{-1}$) intensity. Maximum cell yield was significantly different at low light between the two *Microcystis* species but not between the two strains of *M. ichythyoblabe,* indicating the presence of species-specific differences, but not location-specific differences. In the nutrient experiments, nitrogen (N) was the more important nutrient with little growth under low N treatments in all three strains. Increased concentrations of phosphorous (P) led to higher cell yield when coupled with high concentrations of N. Maximum growth rate for the three strains did not differ significantly, but maximum cell yield was much higher in *M. ichythyoblabe* from Tengeh Reservoir than in *M. ichythyoblabe* and *M. flos-aquae* isolated from Lower Peirce Reservoir. This study is the first to examine growth of *M. flos-aquae* and *M. ichythyoblabe* isolated from tropical reservoirs under varying light and nutrient treatments.

P-100

Control of cyanobacterial bloom and nutrient reduction by periphytic algae

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Although lots of studies have been undertaken on cyanobacterial blooms, this problem has not been solved. The fundamental solution is simple: no nutrient input into water bodies. However, this requires a big budget and a long time to take effect. Nutrients accumulated in the sediment can be released into water for years, even after external sources are completely blocked. Removal of indigenous nutrients still needs to be continued. Periphytic microalgae can give some solutions. When supporting materials are installed in surface water for periphytic algae to attach, periphytic microalgae will take up nutrients that may be used by cyanobacteria. Competition between cyanobacteria and periphytic algae would reduce the extent of bloom formation. Periphytic microalgae growth in the surface water also prevents light penetration into deeper water, interfering with the underwater photosynthesis. Periphytic microalgae could be cultivated even in cold seasons, with larger biomass, thereby reducing eutrophic level further, all year round. Four mesocosms were constructed with different amounts of supporting materials, in Daechung Reservoir, Korea, in late summer 2013. *Microcystis*, *Oscillatoria* and *Anabaena* were dominant during the cultivation period. Cyanobacterial growth was reduced to 1/3 of the control (without supporting materials) by the growth of attached microalgae. Microcystin concentration decreased along with a sharp decline in the cyanobacterial cell density. The highest biomass productivity of periphyton was 0.111 g m^{-1} d^{-1} and the maximum removed concentration of total phosphorus was 85 µg -P L^{-1}. In conclusion, this periphytic cultivation system demonstrated a good performance for nutrient reduction and bloom control.

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Impact of brownification on harmful phytoplankton in Scandinavian lakes

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In the last decades, watercolour has increase drastically in Scandinavian lakes due to increase leakage of terrestrial humic substances. The increase of watercolour (brownification) has led to important changes in the light climate for the phytoplankton. In addition, the leakage of humic substances can potentially provide a new source of carbon for mixotrophic phytoplankton species. It has been hypothesized that *Gonyostomum semen*, considered as invasive and harmful, will become dominant in lakes with browner water, whereas toxic cyanobacteria will be favored in clear waters. To test this hypothesis we experimentally investigated the effect of brownification on phytoplankton communities, with a focus on harmful species (toxic cyanobacteria and the harmful raphidophyte *Gonyostomum semen*). We performed mesocosm experiments in three lakes to simulate the effect of future increases of watercolour (4 concentrations of humic substances). In addition, 20 lakes were sampled along gradients of nutrients and watercolour. Amplicon sequencing of the 16S rDNA gene and qPCR were use to identify changes in phytoplankton community composition.

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Maximising the value of micro-algae in the CICCM

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Micro-algae maintained in the Cawthron Institute Culture Collection of Micro-algae (CICCM) underpin research within Cawthron and globally. One of the goals when adding new species to the collection is the reliable sourcing of target compounds, for example, purified standards for chemical analyses. Suitable isolates from environmental samples are fully characterised using molecular tools and chemical tests and a body of information is then attached to each isolate.
A recent research focus has been the isolation of tropical and sub-tropical species from the Pacific region, including dinoflagellates in the ciguatera fish poisoning (CFP) complex. As a result, the CICCM now maintains maitotoxin (MTX) producing isolates of *Gambierdiscus yasumotoi* from New Zealand, *G. pacificus* from Hawaii and the Cook Islands, and *G. australes* from the Cook Islands. A ciguatoxin producer, *G. polynesiensis*, has also been isolated from the Cook Islands. These isolates are being mass cultured for production of CFP compounds.
Species of *Ostreopsis*, the producer of palytoxin-like compounds, are also being cultured to generate enough material for the characterisation of the toxins produced. For example, *O. siamensis* and *O. ovata* isolates from New Zealand and the Cook Islands vary greatly in their production of palytoxin-like compounds and may even be non-toxic. Palytoxin is of concern in Europe and may become a regulated marine toxin. If this occurs, the toxin standards and rapid test methods needed are available at Cawthron.

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CODIMAR Collection of Marine Dinoflagellates: a decade supporting HAB research

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The *Collection of Marine Dinoflagellates* (*CODIMAR*) is housed in a new facility that is part of the *Laboratory of Taxonomy and Ecophysiology of Marine Microalgae* at CIBNOR. Since February 2004, CODIMAR has been a service collection facility that provides strains for research and education (http://www.cibnor.mx/en/research/biological-collections/codimar). It maintains harmful microalgae species, mainly from the Gulf of California. Presently, CODIMAR has 157 dinoflagellate strains belonging to the orders: Gymnodiniales, Gonyaulacales, Prorocentrales, and Peridinidiales. Curation of rhaphidophyte strains with ichthyotoxic qualities has also been accomplished. The strains had been used in national and international HAB research projects, ongoing studies on growth kinetics, toxicity, taxonomy, genetic characterization, and harmful effects on marine fauna. Investigations using strains from our collection have been used by students in master's and doctoral theses, which have, so far, resulted in 23 scientific publications. The most studied strains have been those of *Gymnodinium catenatum* and *Cochlodinium polykrikoides*. Strains have also been used in taxonomy and biotechnology training courses. CODIMAR is formally listed in the *Public and Private Registry for Museography and Scientific Collection of Wild Species*. We recently completed archiving of the database (project JC006) to formalize its registration in the *National Biodiversity Information System* of the *National Commission for the Knowledge and Use of Biodiversity* (CONABIO) of Mexico as an agency that maintains scientific collections of pests that affect human health. The funding received for this project has helped to improve curatorial quality of strains and renew infrastructure, which ensures operability for the next decade.

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Intra-population variability in allelopathic activity of the bloom-forming *Alexandrium ostenfeldii* and response of co-occurring dinoflagellates

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The PSP toxic, allelopathic dinoflagellate *Alexandrium ostenfeldii* forms dense, recurrent late summer blooms in shallow coastal areas around the Baltic Sea. We studied the intra-population variability of its allelochemical potency and the responses of co-occurring and potentially competing dinoflagellates to the allelochemicals.
The lytic activity of 10 *A. ostenfeldii* strains originating from the northern Baltic Sea was evaluated by their EC_{50} values (i.e. the cell concentration yielding a 50% decline in cryptophyte density), which were found to vary between 236 and 1726 cells mL^{-1}. When sympatric dinoflagellates *Kryptoperidinium foliaceum, Levanderina fissa* and *Heterocapsa triquetra* were exposed to filtrate of *A. ostenfeldii*, short-term (<1h) responses of all target species after an immediate immobilization were species-specific. Almost all of the *K. foliaceum* cells formed cysts, whereas *L. fissa* cells lost their shape and lysed, and *H. triquetra* cells shed their thecae. After 24h, *K. foliaceum* had returned into vegetative cells and the number of immotile *L. fissa* and *H. triquetra* cells had significantly decreased.
The results suggest that coexisting dinoflagellates may develop efficient means to escape and recover from extracellular metabolites produced by highly allelopathic phytoplankton species such as *A. ostenfeldii*.

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Intraspecific diversity and distribution of the cosmopolitan species *Pseudo-nitzschia pungens* (Bacillariophyceae)

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The diatom *Pseudo-nitzschia pungens* is distributed throughout the world's oceanic and coastal environments. We studied 15 *P. pungens* clones from various geographical locations, and these were clearly divided into the three known genotypes (clades) based on internal transcribed spacer sequencing. Clade III is reported for the first time in Korean coastal waters and the East China Sea, and two subgroups were identified. We carried out physiological tests on nine clones of each clade grown under various culture conditions. In temperature tests, the optimal growth range (R_{op}) of clade I clones was wider (R_{op}, 20.1 to 32.9ºC) than that of clades II and III. Clade II clones were considered to be adapted to lower temperatures (R_{op}, 15.1 to 22.9ºC) and clade III to higher temperatures (Rop, >23.7ºC). In salinity tests, clade I clones were regarded as stenohaline (R_{op}, 21.1 to 35.8 psu) and clade II and III clones were euryhaline (R_{op}, 20.5 to 29.5 and 20.3 to 29.0 psu, respectively). In irradiance tests, clade II clones favored relatively lower light conditions (R_{op}, 102 to 243 µmol photons · m^{-2} · s^{-1}) than clades I and II (R_{op}, 107 to 261 and 108 to 257 µmol photons · m^{-2} · s^{-1}, respectively). This supports the hypothesis that *P. pungens* clades have different adaptive strategies based on their places of origin. We also found ultrastructure differences in the number of fibulae, striae and poroids that separate the clades. Our data show that physiological and morphormetric features are correlated with genetic intraspecific differentiation in *P. pungens*.

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Mating compatibility, encystment and excystment characteristics of *Alexandrium catenella* from Chilean southern fjords.

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Resting cysts have been proposed to play an important role in the origin and initiation of *A. catenella* blooms in the seasonally variable Chilean southern fjords. However, it remains unclear whether endogenous or environmental mechanisms regulate the encystment timing and rate of Chilean strains. We investigated mating compatibility among 10 Chilean Group 1 *A. catenella* strains using the approach of Blackburn et al. (2001). Strains were crossed in all possible pairwise combinations and excystment rates assessed under a range of abiotic variables. Reproductive compatibility was highly variable among strains with high compatibilities among northern (Los Lagos) and southern (Magallanes) but not central (Aysén) strains. Encystment was synchronized in all pairwise crosses, with cyst production starting 26 days and terminating 45 days after inoculation. Cyst production was highly variable among crosses ranging from 7±1.3 to 316.9±20.5 cysts ml^{-1} (400 cell ml^{-1} as inoculum). The dormancy period was estimated to be between 80 and 120 days with a maximum cumulative excystment of 60%. The following conditions enhanced excystment rate: Low temperature (10-12°C), high salinity (30 psu), N and/or P depletion, and low irradiance (20 µE m^{-2} s^{-1}). These results suggest cysts may play a more important role in seeding *A. catenella* blooms than previously considered. The precise location of cyst beds need to be further defined.

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Resting cysts, and effects of temperature and salinity on the growth of vegetative cells of the potentially harmful species *Alexandrium insuetum* Balech (Dinophyceae)

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The potentially harmful species *Alexandrium insuetum* was studied by the incubation of resting cysts isolated from sediment trap samples collected at Jinhae-Masan Bay, Korea. They were characterized by morphological and phylogenetic analysis. The effects of temperature and salinity on the growth of *A. insuetum* were also investigated. The resting cysts were characterized by a spherical shape, a small size (20-25 µm) and the presence of either three or four red accumulation bodies. The similarity of morphological features of the resting cysts to those of other species of the minutum group (consisting of *Alexandrium minutum* and *A. tamutum*) indicates that the morphological features of resting cysts might improve the accuracy of the grouping of *Alexandrium* species. *A. insuetum* germinated from the resting cysts and was morphologically consistent with vegetative cells reported from Korean and Japanese coastal areas, and had a partial large subunit (LSU) rDNA sequence identical to that from Japanese strains. The growth of *A. insuetum* was observed between 20 and 35 psu, with increasing temperature; however at 25°C, *A. insuetum* grew even at 15 psu. The highest growth rate (0.60 d^{-1}) was observed at 25 °C and 25 psu, which is higher than the previously reported growth rate of *A. tamarense,* which is responsible for outbreaks of paralytic shellfish poisoining and blooms in Jinhae-Masan Bay. These results suggest that the proliferation of *A. insuetum* in Jinhae-Masan Bay is likely to be highest during the summer.

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Which is the real resting cyst of *Cochlodinium polykrikoides* Margalef (Gymnodiniales, Dinophyceae)?

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The ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* Margalef is the most notorious causative species of dense blooms that have occurred annually in Korean coastal waters. These dense blooms have caused major economic losses to the aquaculture industry: USD $7 million in 1993, USD $60 million in 1995 and USD $4-19 million in 2000-2003, 2007 and 2012 (NFRDI, 2012). For this reason, intensive research projects funded by the Korean government were initiated to clarify the mechanisms behind the outbreak of *C. polykrikoides* blooms. However, most of these research projects have concentrated exclusively on understanding the introduction and origin of vegetative cells of *C. polykrikoides*. This is because Matsuoka et al. (2010) reported that the outbreaks of dense blooms in Asian coastal areas, such as in Korea and Japan, might be related to the transportation of vegetative cells of *C. polykrikoides* by the Tsushima Warm Current, and the researchers suspected that the resting cysts of *C. polykrikoides* are present in natural sediments, despite the fact that the production of resting cysts by *C. polykrikoides* has been confirmed by laboratory experiments. This study provides the morphological features of resting cyst of *Cochlodinium polykrikoides* collected from natural sediments in Tongyeong coastal area, Korea. The evidence for resting cyst of *C. polykrikoides* is provided using the morphological and phylogenetic data of cells germinated from this cyst. The morphology of resting cyst differs from that previously reported in sediments and culture experiments. The distinct feature is that the cyst body is covered by the reticulate ornaments and spines.

P-109

Dinoflagellate cysts from the Eastern Adriatic Sea

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Among 2000 known marine dinoflagellate there are about 200 species produce cysts as part of their life cycle. These benthic phases allow them to survive during unfavorable conditions and reinoculate the water column when favorable conditions are restored. In order to detect non-indigenous and invasive species for the first time dinoflagelate cysts was determined in the eastern Adriatic Sea. Sediment samples were collected in Kaštela Bay in 2011 and Šibenik Bay in 2013. The samples were prepared using standard palynological procedures. The granulometric composition of the sediment as well as the proportion of organic matter in sediment was determined. In the Kaštela Bay 24 dinoflagelate cysts were determined out of which 13 belong to Peridiniales, 6 belong to Gonyaulacales, 4 belong to Gymnodiniales and 1 belongs to Prorocentrales. The most abundant were cysts of *Lingulodinium polyedrum* with abundance of 10,370 cysts g^{-1} dry sediment followed by *Alexandrium minutum* with abundance of 250 cysts g^{-1} dry sediment. The highest abundances of dinoflagellate cysts were recorded at station with the finest sediment mostly comprised from silt and clay. In the Šibenik Bay 12 dinoflagellate cysts were determined. Cysts of dinoflagellate *A. minutum* were the most abundant with 130 cysts g^{-1} dry sediment. Our study of dinoflagellate cysts from surface sediments revealed presence of viable cysts of harmful dinoflagellate, which could occupied the water column if optimal condition for its growth occured.

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Cyst morphology, germination characteristics, and toxicity of *Pyrodinium bahamense* in the Gulf of California

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Pyrodinium bahamense is a meroplanktonic dinoflagellate that produces toxins that cause paralytic shellfish poisoning when populations explode in algae blooms along tropical and subtropical coasts. Cyst morphology, germination characteristics, and toxicity were investigated, using samples from Isla San José in the Gulf of California. Morphology was observed with light and scanning electron microscopy. Germination was tested at different temperature (15–35 °C), salinity (15–35), and culture media (natural seawater, GSe, and f/2). To determine levels of toxicity of vegetative cells grown after cyst germination, nine strains were subjected to toxin analyses by fluorescence HPLC. Morphological features and size of cysts are in general agreement with previous descriptions, particularly morphotypes found in the subtropical North Atlantic, where *P. bahamense var. bahamense* occurs. Cysts exhibit thermophilic and euryhaline characteristics. Highest germination occurred at 20–35 °C, with a peak range at 25–30 °C. Excystment occurred at salinities from 20–35. Germination occurred in the three culture media, but was highest under optimal temperature range in the culture medium that included terrestial soil extract and selenium. Only one strain exhibited toxicity; saxitoxin was detected in high concentrations (95 pg STX eq cell^{-1}).

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Cyst abundance and excystment variation in *Alexandrium catenella* from inner sea of southern Chile

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Several ecological studies in *Alexandrium* blooms have demonstrated that is critical to know the requirements for cyst germination (endogenous control and/or environmental driving) to understand the ability of germling cells to contribute to population dynamic and the initiation of the blooms. Further, modeling and observational studies have showed that also the size of the cyst population in the sediment can play a decisive role in the special and temporal bloom variation. In this study, we have evaluated the distribution of cyst at inner sea bay (Chiloé) affected by *Alexandrium* blooms and the factors affecting excystment process. In this bay 17 sites were monitored and cysts, delivered from this place during different years, were evaluated for germination under distinct conditions in laboratory. The cyst abundance was scarce and spatially heterogeneous (ranging from 0 to 6.6 cyst/mL of sediment). Conversely, the maximum cumulative excystment was not higher than 30% last year that which was considerably minor to 70-80% achieved years before. Under darkness the cyst germination was significantly reduced but not inhibited. Accumulative percentage of germination was improved only at low irradiance (10 µmoles photons m^{-2} s^{-1}), while low temperature (9 °C) did not reduce the maximum accumulative excystment but delaying significantly the timing of germination. These results indicate that cyst germination in *A. catenella* has close linkage to environmental factors, inferring an opportunistic strategy. However, the scarce cyst abundance detected doubts its contribution to the bloom initiation.

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High biomass blooms in beaches: are resting cysts maintained *in situ?*

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Data derived from an extensive sampling (74 beaches in summer 2012) along the Sardinian coasts have allowed the identification of *Alexandrium taylorii*, *Gymnodinium instriatum* and *Barrufeta bravensis* as the most widely distributed cyst-forming harmful species. Statistical analyses indicated that the distribution of harmful algal species was correlated with gravel and medium-fine sand substrata. This relationship suggested that vegetative cells might be recruited from cyst beds in beach sediments. Therefore, the objective of this study was to verify the presence of resting cysts in the near shore beach sediments. Sediment cores (40-50 cm long) were collected in May 2013 from three target beaches. Cores were sampled at three stations (foreshore, trough, bars) along a perpendicular transect at each beach. Cores were sectioned every 5 cm. *Alexandrium taylorii* cysts were observed in the three beaches, always below a depth of 15 cm of sediment. Cyst presence was assessed at the trough and bar stations in Platamona and Villasimius, whereas at the foreshore station in Bosa. *Gymnodinium instriatum* cysts were detected only in Bosa at the bar station, at a depth below 20 cm. Naked-like dinoflagellate cysts, probably belonging to *Barrufeta bravensis*, were observed at the same station and depth. The found cyst distributions reflected the different morphodynamic features of the beaches. The discovery of resting cysts in the near shore sediments supports the hypothesis of a potential '*in situ*' source of vegetative cell inoculum.

P-113

Invasions of *Alexandrium minutum* unveiled by sediment biological archives

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Blooms of the potentially toxic dinoflagellate *Alexandrium minutum* are frequently recorded by local monitoring networks along the Brittany coasts (Western France) since the end of the 1980's. Several authors consider this species invasive due to its absence in ancient phytoplankton inventories. However it is hard to state only on the basis of monitoring network data series (<40 years) whether the species is indigenous (initially present at low concentrations and suddenly able to proliferate) or invasive (imported from abroad). The analyses of dinoflagellate biological traces preserved in sediments (cysts, DNA) could be an alternative strategy to trace back the history of an invasion (paleoecological approach) and to evaluate its actual spatial extension. We sampled and dated (^{210}Pb and ^{137}Cs chronology) sediment cores (<40 cm) from the Bay of Brest and our sediment chronology allowed going back to 1939±2. Genetic data (real-time PCR on the ITS rDNA region) and germination experiments showed that the abundance of the species increased in the last 90's, corroborating national motoring plankton data. Moreover, in 2013 analyses of superficial (<3 cm) sediments of 30 stations from the Bay of Brest proved that at present *A. minutum* spread across the whole bay, while literature data of the area documented that its cysts were absent in 1990. The history of *A. minutum* invasion in Brittany will be reconstructed at mesoscale (<300 km costal area) comparing sediment cores of sites characterized by different anthropogenic impact, bioclimatic regimes and where intense blooms were reported before the 1990's.

P-114

Influence of riverine DOM on a coastal plankton community from the Baltic Sea

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Nitrogen is the potential limiting factor for the primary production in the Baltic Sea, especially during the summer. The effects of riverine DOM nitrogen and inorganic nitrogen input on a natural plankton community which was collected from the surface water of the near shore of Kalmar Sound, Sweden on August 9th, 2011 were compared under a batch culture experiment. The inorganic nitrogen input favoured the growth of pico-diatoms, and large bacteria and ciliates whereas the riverine DOM nitrogen input favoured the growth of pico-flagellates and ciliates and a smaller size of bacteria and ciliates. Results suggested that the riverine DOM can be an important nitrogen source in the southern coastal waters of the Baltic Sea. The input of riverine DOM into the coastal seawater will promote the growth of flagellates and ciliates and induce a size miniaturization of the microbial loop.

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Viruses infecting harmful bloom forming diatoms in western Japan

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Diatoms are one of the siginificant primary producers in coastal environments. Diatom blooms can cause considerable fisheries damage. Cultivation of the seaweed laver (*Porphyra tenera*) is of significant economic importance in Japan, however, diatom blooms have often caused depletion of nutrients and damaged the seaweed cultures due to discoloration of the thalli. Therefore, it is essential to reveal the dynamics of diatom blooms and its related factors. Viruses in aquatic environments are now considered to be one of the most significant factors to control microalgal populations as well as chemical and physical factors, especially during bloom period. Previous studies showed the existence of viruses infecting diatoms.To date at least 12 diatom viruses have been isolated and characterized. These viruses are grouped into two categories based on genomic features, single-stranded (ss) RNA and ssDNA. So far, five different ssRNA diatom viruses have been reported, and their hosts include genera *Rhizosolenia*, *Chaetoceros*, and *Asterionellopsis*. Very recently, an ssRNA virus infecting *Thalassiosira rotula* was isolated. They harbor ca. 9kb ssRNA genome with two open reading frames encoding putative replication-related proteins and capsid proteins. As for ssDNA diatom viruses, six different species have been characterized, and their host genera includes *Chaetoceros* and *Thalassionema*. Their genomes are composed of ca. 6kb circular ssDNA. These diatom viruses are all lytic to their respective host diatoms; and their infection is strain-specific. These discoveries are important to understand diatom ecology.

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Induction of virus resistance in planktonic diatom *Chaetoceros tenuissimus* under co-culture with bacteria

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Phytoplankton dynamics in nature respond to various environmental factors (e.g., water temperature, salinity, light, nutrients, and water movement). Viral impact, while, is also considered to be important for understanding the phytoplankton dynamics. Generally, host populations are not completely killed by viruses in natural environments. In contrast, viruses kill the host cells almost completely under culture conditions. To date, it has been unclear the reason of co-presence between host plankton and virus in natural waters. The virus CtenRNAV is a lytic virus infectious to the marine planktonic diatom *Chaetoceros tenuissimus*. In this system under an axenic condition, the host cultures completely crashed due to viral infection. Under the presence of a bacterial community, while, the host cultures survives through CtenRNAV infection. We isolated a subclone from the survivors and used for further experiments. Although this subclonal culture was permissive for CtenRNAV infection during an exponential growth phase, but not allow the viral proliferations in a stationary phase. The dominant groups in the bacterial community were *Nautella* sp., *Sulfitobacter* sp. and *Polaribacter* sp., all of which contributed to viral resistance. Ultrastructural observations showed that these bacteria seemed to contact to diatom cells closely, however, the mechanisms to drive the viral resistance of the host culture were uncleared in the present experiments. Further analysis of the relationships among microalgae, viruses and bacteria, may contribute to understand the phytoplankton dynamics in nature.

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Viral dynamics in relation to bacterial and cyanobacterial hosts in a subtropical reservoirs system, Queensland, Australia

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Relationships between viruses, their hosts and the environment have seldom been investigated in sub-tropical freshwaters. In an Australian sub-tropical reservoir system we sampled viruses and their potential hosts (heterotrophic bacteria, cyanobacteria, eukaryotic algae, along with physiochemical variables, from the austral spring 2008 to autumn 2009 (nine months). In the reservoirs, potentially toxic cyanobacteria commonly bloom and dominate the phytoplankton community during the warmer months, accounting for no less than 74% of the total phytoplankton abundance. The total number of viruses ranged from 0.2 to 32.4 $x10^7$ mL^{-1} (average 6.4 ± 0.5 $x10^7$ mL^{-1}). Viral abundance positively correlated with total bacterial counts, as most viruses in the reservoirs are likely from bacteria. While viruses did not correlate with the total cyanobacterial abundance or chlorophyll *a,* they were significantly negatively correlated with two cyanobacteria genera; *Aphanocapsa* and *Microcystis*, which could potentially be a result of viral induced mortality influencing host species population dynamics.

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Isolation and characterization of new viruses infectious to harmful bloom-forming microalga dinoflagellate *Karenia mikimotoi*

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Karenia mikimotoi is harmful bloom-forming microalgae mainly in western Japan and causes mortality of fish and shellfish. Three viruses infecting the dinoflagellate *K. mikimotoi* were isolated from the coastal water of Ago Bay, Japan, in July 2013. All viral particles found in this study were icosahedral, lacking tail, and 80 nm with double-stranded DNA. The viruses caused lysis for 5 – 10 days their host strains at 20 ˚C under 12-h light-dark cycle; the light (130 to 150 µmol of photons $m^{-2}s^{-1}$) was provided by cool white fluorescent illumination. Host ranges of the viruses examined among 20 *K. mikimotoi* strains were widsh, i.e., each virus infected 7-8 strains including the host used for isolation. Recently, the virus is expected to be a promising tool for preventing the occurrence of some harmful microalgae. In fact, *Heterocapsa circularisquama* and its ssRNA virus (HcRNAV) had showed synchronous fluctuation pattern in abundance in natural environments, the virus infection has been considered to have a significant impact on the host bloom dynamics. In this report, we will introduce the viral effects on *K.mikimotoi* blooms and potential as a preventing tool in natural environment as well as the characteristics of the new viruses.

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Characterization of a cyanobacteriolytic bacterium and its lytic mechanism

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Noxious cyanobacterial blooms are observed in many eutrophic lakes worldwide, and sometimes cause the deaths of livestock and wild life and create serious problems for freshwater management. However, the precise mechanism of the blooms dynamics is not clarified yet. The blooms sometimes decreased in cell number when cyanobacteriolytic bacteria increase. These bacteria may play an important role at the occurrence and ending of the blooms. In this study, we report a novel rod-shaped bacterium with high cyanobacteriolytic activity from Lake Hirosawa, Kyoto, Japan, using *Planktothrix agardhii* in a screening system. It lysed wide range host strains including *P. agardhii*, *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*. Phylogenetic analyses based on 16S rRNA gene sequence suggested that it might belong to a novel genus with in family *Cytophagaceae*. The lytic activity of the bacterium to *Planktothrix agardhii* NIES104 was observed in a fraction of molecular weight between 50,000 and 100,000 of the bacterial culture. Moreover, it was inhibited by trypsin and chymotripsin inhibitors. These results suggest strongly that proteolytic enzyme(s) might play an important role in the lytic process.

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High density of algicidal and growth-inhibiting bacteria against harmful algal species detected from seagrass and macroalgae in Puget Sound

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The spatial distribution of algicidal and growth-inhibiting bacteria associated with seagrass and macroalgae were investigated throughout Puget Sound, WA, USA. During the summer of 2012, we sampled the leaves of *Z. marina* and adjacent seawater from 14 sites in the Puget Sound. In 2013, we extended our survey to include samplings of other seagrass species as well as the water column surrounding various macroalgal species. The high densities (2.7×10^6 CFU g wet leaf^{-1}) of *H. akashiwo*-killing bacteria and of *A. tamarense* growth-inhibiting bacteria (8.3×10^6 CFU g wet leaf^{-1}) were both detected from *Z. marina* collected from north Padilla Bay in 2012. AB and GIB against *H. akashiwo* were successfully isolated from 3 different species (*Z. marina*, *Z. japonica* and *Ulva lactuca*) in 2013. *H. akashiwo*-killers were found from *Z. marina* (1.0×10^8 CFU g wet leaf^{-1}) and the green algae *U.lactuca* (1.3×10^8 CFU g wet weight^{-1}) collected from Shallow Bay, Sucia Island and the highest density of *H. akashiwo*-GIB (2.8×10^8 CFU g wet weight^{-1}) were detected from *Z. japonica* leaves collected from Padilla Bay. These findings provide a new prospective for the protection of seagrass and macroalgae beds that provide habitat for AB and GIB as a means to prevent or mitigate HABs. We will also discuss about our new project comparing seasonal variability of algicidal bacteria at two different seagrass beds in Puget Sound.

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Isolation and identification of a marine anti-algal bacterium and mechanisms of inhibitory effect on *Phaeocystis globosa*

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The bloom of *Phaeocystis globosa* has occurred frequently in the southern coast of China in recent years, which has led to substantial economic losses. In this study, a bacterial strain Y4 which is capable of inhibiting the harmful algae *P. globosa* was isolated from coastal surface water in Zhuhai, Guangzhou Province, China. The isolated strain was identified as *Bacillus* sp. by culture morphology, biochemical reactions, and homology research based on 16S rDNA. The algicidal effects were assessed by means of the analysis of its pigment content after 96 h exposure to culture filtrate of strain Y1 and Y4, using HPLC, and by means of flow cytometry coupled with the PI stain and chlorophyll-a auto-fluorescence, which was used to quantify and compare the effect of strain Y1 and Y4 on cell integrity for the tested *P. globosa*. Results showed that chlorophyll fluorescence intensities and cell integrity decreased with time of exposure to culture filtrate, and the percentage of live cells was 2.9% and 24.9% after 96 h of exposure to strain Y1 and strain Y4, respectively. These findings suggested that algicidal bacteria led to pigment degradation and membrane integrity damages. This is the first time that flow cytometry is used to characterize and quantify the effect of bacterial culture against *P. globosa*.

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A serial control of two different *Amoebophrya* parasites on *Cochlodinium polykrikoides* blooms in the southern Korean coastal waters

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There are technical limitations to conducting ecological studies of the parasite *Amoebophrya*, due to difficulties in observation via light microscopy. To overcome this issue, we developed a qPCR assay for *Amoebophrya* species in southern Korean coastal waters.
Specific primers for two *Amoebophrya* species were designed to target the internal transcribed spacer (ITS). To establish a standard curve, target PCR products were cloned into a pCR2.1 vector using a TA cloning kit, and then transformed into *E. coli*. After the extraction of plasmid DNA, the copy number of each sample was calculated and serial dilution was performed in order to construct a standard curve. The C_t value and the log of the copy number showed a significant linear relationship ($r^2 \geq 0.99$). *C. polykrikoides* blooms occurred twice at St. Mulgun Harbor, in August and October of 2012. Using the real-time PCR assay, the free-living cells showed similar dynamics to those during their parasitic stage living inside host cells. While *Amoebophrya* sp. 1 was predominant in August, *Amoebophrya* sp. 2 was strongly related with the termination of the *C. polykrikoides* bloom in October. Interestingly, the copy number of *Amoebophrya* sp. 1 in the parasitic stage was high, whereas that of the dinospore was extremely low on 16th October, even though *C. polykrikoides* had sharply increased. This implied that *Amoebophrya* sp. 1 was capable of infecting host cells, but could not develop into the trophont or vermiform stages. Accordingly, these two *Amoebophrya* species are thought to play an important role in terminating *C. polykrikoides* blooms in southern Korean coastal waters.

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The potential for arms race and Red Queen coevolution in a protist host-parasite system

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The dynamics and consequences of host-parasite coevolution depend on the nature of host genotype-by-parasite genotype interactions (G×G) for host and parasite fitness. G×G with crossing reaction norms can yield cyclic dynamics of allele frequencies ('Red Queen' dynamics) while G×G where the variance among host genotypes differs between parasite genotypes results in selective sweeps ('arms race' dynamics). Here we investigate the relative potential for arms race and Red Queen coevolution in a protist host-parasite system, the dinoflagellate *Alexandrium minutum* and its parasite *Parvilucifera sinerae*. We challenged 9 different clones of *A. minutum* with 10 clones of *P. sinerae* in a fully factorial design and measured infection success and host and parasite fitness. Each host genotype was successfully infected by four to ten of the parasite genotypes. In bottles where infection was successful, the fitness (growth rate) of the host was severely reduced compared to un-inoculated controls. In contrast, in bottles where infection was unsuccessful, host growth rate was higher than in uninoculated controls. There were strong G×Gs for infection success, as well as both host and parasite fitness. About 3/4 of the G×G variance components for host and parasite fitness were due to crossing reaction norms. There were no general costs of resistance or infectivity. We conclude that there is high potential for Red Queen dynamics, but also some scope for selective sweeps in this system.

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Toxic effects of *Vicicitus globosus* (Class Dictyochophyceae) and *Chattonella marina* (Class Raphidophyceae) on other microalgae and rotifers

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Cultures of *Vicicitus globosus* (previously *Chattonella globosa*) and *Chattonella marina*, established during the 2010 fish kill event in Mahanga Bay, Wellington Harbour, are confirmed to be toxic. The aggregate toxicity of lipophilic cell extracts of each species were evaluated using three species each of flagellates, dinoflagellates and diatoms, and a rotifer as test organisms. Cell extract of *V. globosus* was found to destroy cells of all nine microalgae, in a matter of a few minutes to less than 20 minutes, while that of *C. marina*, was found to destroy all species over 10 to 30 minutes. Tests conducted on rotifers showed the same trends; animals exposed to a cell extract of *V. globosus* died in a much shorter time (Lt50 = 80 minutes) than those exposed to an extract of *C. marina* (20 hours).

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Effects of toxic dinoflagellate *Alexandrium catenella* (Whedon & Kofoid) Balech 1985 on naupliar development of calanoid copepod *Acartia tonsa* (Dana, 1846)

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Zooplankton is a key link between phytoplankton and higher consumers in marine food web. Here we investigate the effect of several diets of toxic *Alexandrium catenella* on the naupliar development of the copepod *Acartia tonsa*, assessing the effects over time on the larval development and the survival rates of nauplii exposed to both toxic and non-toxic diets (*Tetraselmis suecica*). The nauplii used in this study were collected in two different areas (Agua Fresca (AF) and Chabunco Bay (CB)) of the Magellan Strait (53°S), with different historical exposition records to *A. catenella* blooms. The experiment was carried out in six days, were both *A. tonsa* populations were feed with toxic and non-toxic algae, at two experimental food concentration (low concentration, ~ 400 µg C L^{-1} and high concentration ~ 800 µg C L^{-1}). The results showed that at low concentration food, the nauplii population of AF had higher survival rates (~ 40%), versus the CB population (<25%); on high concentration food, both locations showed a low nauplii survival rate, which indicates that there was no differential effect on the consumption of *A. catenella*, probably due the high concentration food available on the environment. The findings observed in low concentration food trials, suggest that *A. tonsa* nauplii showed a differential tolerance to the toxin consumption on the population historically exposed to the algae and, at the same time, decreasing the survival and reducing the early larval stages in those populations without historical records of exposure to the toxic algae.

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Toxic diatoms in the arctic marine food chain: The effects of domoic acid on copepodites of arctic *Calanus* copepods grazing on the diatom *Pseudo-nitzschia seriata* in Disko Bay, West Greenland

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Copepods of the genus *Calanus* dominate the mesozooplankton in arctic waters. The copepodite stages of *Calanus* are the most abundant zooplankton during phytoplankton spring blooms, and are therefore ecologically important in arctic waters. However, only a few studies have explored their grazing on phytoplankton. It has been shown that grazing of the adult female *Calanus* copepods on the domoic acid (DA) producing diatom *Pseudo-nitzschia seriata* results in accumulation of DA in their tissue, and effects on their temporal grazing pattern have been indicated. In this study we explored if the arctic *Calanus* copepodites accumulate DA when fed toxic *P. seriata,* and whether this affected their mortality and grazing rates. The study took place in Disko Bay, West Greenland where *Calanus* spp. in developmental stages III and IV were collected. To determine the temporal grazing pattern, the copepodites were fed with different concentrations of *P. seriata* and the grazing rates were measured for 39 h. The grazing rates were compared to the grazing on the non-toxic *Pseudo-nitzschia obtusa*. Furthermore, it was studied whether the copepodites could discriminate between toxic and non-toxic *Pseudo-nitzschia* species when fed mixed cultures. Accumulation of DA in the copepodites was measured in the end of the experiments. To explore if the grazing by the copepodites could affect the DA production of *P. seriata*, unialgal cultures were exposed to different copepodite concentrations, and DA levels were measured. To date, no knowledge of the phycotoxin effects on copepodites of the arctic *Calanus* exists.

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The ability of the marine diatoms Pseudo-nitzschia multiseries and Pseudo-nitzschia pungens to inhibit the growth of phytoplankton via allelopathy

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Diatoms within the genus *Pseudo-nitzschia* can form near-monospecific blooms in both natural and iron-fertilized high-nutrient, low-chlorophyll (HNLC) regions and can have detrimental impacts on marine ecosystems. Here we demonstrate the ability of *P. pungens* isolated from the South China Sea and two strains of *P. multiseries* isolated from the Bay of Fundy, Canada, to produce extracellular compounds capable of lysing and/or inhibiting the growth of multiple phytoplankton species. Since the allelopathic activity was found in both *P. multiseries*, which produces domoic acid (DA), and *P. pungens*, which produces little if any DA, the allelopathic effects of *Pseudo-nitzschia* spp. seem to be unrelated to DA. Allelopathic inhibition of other phytoplankton was documented during exponential and stationary growth phases of *Pseudo-nitzschia* and the strongest allelopathic effects were obtained from sonicated cultures, suggesting that the sudden release of allelochemicals via processes such as cell lysis or zooplankton grazing may have the strongest effect in an ecosystem setting. Differences in the responses of target species to *Pseudo-nitzschia* spp. suggest these algae may produce multiple compounds that vary in their allelopathic potency and composition as a function of species, strain, growth stage, and perhaps other factors. Collectively, these results suggest that the allelopathy may affect competition between *Pseudo-nitzschia* spp. and other phytoplankton and may play an important role in the formation and persistence of natural and iron-fertilized blooms.

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Toxicity, not morphology, is the dominant defence of the saxitoxin producer *C. raciborskii* against copepod grazers

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Toxicity and morphology are considered to be the main defense mechanisms of bloom forming cyanobacteria against zooplankton grazing. Yet, the relative importance of each of those factors remains poorly known. We tested the effects of chemical and morphological defenses of the bloom-forming cyanobacterium *Cylindrospermopsis raciborskii* on the feeding response of the selective calanoid copepod *Eudiaptomus gracilis*, using a saxitoxin producing (STX+) and a non-saxitoxin (STX-) producing strain as food. From each of these two chemotypes, we created three different morphotypes of contrasting filaments lengths by incubating the strains at 17, 25 and 32°C. We hypothesized that the inhibitory effects of saxitoxins would determine avoidance to *C. raciborskii*, and that morphology would only be relevant in the absence of saxitoxins. Among STX+ strain cultures, the saxitoxin content per biomass unit increased strongly with higher temperatures, while the relative contribution of different variants also changed. Copepods strongly reduced ingestion on the STX+ strain regardless of filament size. In contrast, small filaments of the STX- strain were significantly more consumed than longer ones. The avoidance of all STX+ treatments could indicate that saxitoxins acting as the major anti-grazer mechanism to selective copepods and morphology seems to only have a similar effect in the absence of saxitoxin. *C. raciborskii* intraspecific variation to grazing vulnerability may facilitate its success in diverse environments and regions. Temperature increase will probably enhance this variability because of its direct influence on the morphology and toxicity of this species.

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Grazing of *Aureococcus anophagefferens* by *Pseudodiaptomus poplesia*

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With the objective to elucidate the ecological roles of *Pseudodiaptomus poplesia* in blooming tide, we determined the ingestion rate (IR) of *P. poplesia* on *A. anophagefferens* and *Skeletonema costatum* alone or in the mixture at three life stages under different food levels and then the effects of *A.anophagefferens* on the naupliar development. A typical Michaelis-Menten pattern existed between IR and food level at food concentrations of 0.1-2.0 mg C L^{-1} feeding on both species. Maximum IRs were 1.36, 2.01, 1.90 ug C ind^{-1} day^{-1} for nauplii, copedite and adult stage on *A. anophagefferens*, respectively, and were 1.03, 2.10 and 2.94 ug C ind^{-1} day^{-1} on *S. costatum* sequentially. *P. poplesia* did not show any grazing selectivity in mixed diet of *A. anophagefferens* and *S. costatum*. Mass specific IR were 42.98%-89.40% or 64.02%-68.21% body C day^{-1} feeding on *A. anophagefferens* or *S. costatum* integrating life stages. The growth rate (*g*) of nauplius feeding on *A. anophagefferens* alone was similar to those starved individuals, and survived 7-8 days at nauplii Ⅳ-Ⅴ. *P. poplesia* exhibited significantly lower *g*, reproduction rate, and survival on the mixed diet than those on the diet of *A. anophagefferens* alone. Our results strongly suggested that feeding of *A. anophagefferens* resulted into stunted naupliar development and impaired reproduction in female *P. poplesia*. It was implicated that *P. poplesia* may suppress brown tide potentially and, however, *A. anophagefferens* may not support development of population of *P. poplesia* due to its poor nutrition.

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Analysis of abiotic and biotic factors during the *Noctiluca scintillans* bloom

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Dinoflagellate *Noctiluca scintillans* bloom was recorded at the end of 2002 in coastal waters of the eastern Adriatic, lasting with approximately the same intensity until May 2003. In January and February 2003, similar bloom was observed in the oligotrophic waters of the south Adriatic. This paper discusses the accompanying abiotic (temperature, salinity) and biotic factors (phytoplankton chlorophyll *a*, microzooplankton and net zooplankton), in comparison to available long-term data (temperature, salinity, phytoplankton, microzooplankton, net zooplankton, chlorophyll *a*). Results show that *N. scintillans* bloom was accompanied by the changes in phytoplankton biomass, and atypical occurrences in population structure and density of almost all zooplankton groups. The bloom period was marked by the extreme qualitative and quantitative scarcity of tintinnids and net zooplankton in the northern as well as in the central and southern Adriatic, in addition to domination of poecilostomatoid copepods in the central and southern Adriatic, which was directly related to the presence of *N. scintillans* and other gelatinous organisms. Since *N. scintillans* and the majority of net zooplankton have similar dietary requirements, they are in competitive relationship for food. Therefore, the assumption is that reduced zooplankton abundance enabled the extremely strong proliferation of *N. scintillans*.

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Environmental triggers of programmed cell death in two dinoflatellates *Cochlodinium polykrikos* and *Amphidinimium carterae*

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Numerous laboratory and field studies have investigated the initiation of blooms, but little is known about the causes of bloom decline and termination. Algal death in natural environment is commonly known to occur primarily by grazing, sinking, or cell lysis due to infection with viruses. Another mechanism of cell death is programmed cell death (PCD), which has been receiving increased attention. PCD is an active process that is mediated by changes in morphology, gene expression and protein synthesis inside the cells. This study focus on bloom forming species which might initiate PCD in response to environmental stress, leading to the hypothesis that an active cell death pathway may contribute to the decline of blooms *in situ*. We used high and low temperatures test to induce PCD in *Cochlodinium polykrikoides* and *Amphidinium carterae,* which enabled us to develop methods for detecting PCD markers in unicellular organism, and determine the marker's expression during PCD in these species. We measured PCD markers in *C. polykrikoides* and *A. carterae* under temperature and nutrient stress. Some results show that *C. polykrikoides* does not display clearly the morphology changes by PCD and molecular hallmarks when exposed to 10, 20, and 30^{o}C. Whereas *A. carterae* was temperature-stressed at 10 and 35^{o}C, where it showed the highest proportion of cell exhibiting PCD hallmarks after 24 hour, and died by 72 hour. On the other hand, *A. carterae* did not show PCD hallmarks, but necrosis, when it encountered nutrient stress during a culture experiment with N or P deficiency.

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Exploring the dynamics and feeding behaviour of the Cryptomonad/Ciliate/Dinoflagellate consortia

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Dinophysis sp. is a mixotrophic dinoflagellate that sequesters organelles from other microorganisms. Culturing *Dinophysis* sp.in laboratory conditions relies on the ciliate prey species *Mesodinium* sp., which feeds on the cryptophyte *Teleaulax* sp., which provides the organelles transmitted through this food chain.
We conducted a series of experiments exploring other candidates for the described food chain. Small-scale screening tests, performed in 24-well plates and lasting 2 weeks, were used to identify alternative preys for both *Mesodinium* sp. and *Dinophysis* sp. Micro-algal strains were selected from the Cawthron Institute Culture Collection of Micro-algae (CICCM). *Crysochromulina hirta*, *Crysochromulina simplex*, *Cryptomonas* sp, *Gymnodinium simplex*, *Pavlomulina kotuku* and *Teleaulax amphioxeia* (as control) were tested as alternative prey species for *Mesodinium rubrum*. *Akashiwo sanguinea*, *Alexandrium minutum*, *Amphidinium massartii*, *Gymnodinium simplex* and *Pavlomulina kotuku* were tested as alternative prey for *Dinophysis acuta*.
A second experiment, performed in 50 mL pottles and lasting 7 weeks was performed with preys selected from the screening test for *Mesodinium rubrum*.
The results indicate *Pavlomulina kotuku* and *Crysochromulina simplex* as promising prey species for *Mesodinium rubrum*. In the wells containing *Pavlomulina kotuku*, mucus traps and pigmented *Dinophysis acuta* cells were observed, suggesting sequestration of organelles but this consortia was insufficient to sustain *Dinophysis acuta* growth under these conditions.

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A comparative study of recurrent blooms of *Alexandrium catenella* in two Mediterranean coastal environments over an 11-year period

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Blooms of the toxic dinoflagellate *Alexandrium catenella* (temperate Asian clade) develop every year in two coastal environments in the NW Mediterranean Sea: Tarragona harbour and Thau lagoon. Using an 11-year data series (2000–2010) from these localities, we studied the annual pattern, seasonal variability and trends in *A. catenella* cell abundance. Samples from Thau (2000–2010) were re-examined with the calcofluor technique, revealing that *A. catenella*, not *A. tamerense*, is the species that produce annual recurrent blooms in Thau. We also studied the seasonal variability and the influence of the related biological and physico-chemical variables in the bloom events, and examined the effect of local winds on bloom dynamics.
The occurrence of rhythms in the two *A. catenella* time series was investigated by Fourier spectral analyses, which showed two different frequency patterns: a periodicity of 4 months in Tarragona, and a clear annual bimodal pattern in Thau. There were no relevant trends in *A. catenella* cell abundances in both locations.
The seasonality of *A. catenella* differed at the two sites, although both were subject to two temporally distinct blooming periods (mid-May–end July, mid-August–mid-October in Tarragona; and May–June, Sept–mid-Nov in Thau). This different seasonality at both locations evidenced that *A. catenella* blooms are driven by local factors. Periods of calm wind are required for bloom development at both sites. In contrast to Thau a wind stress previous to bloom development is not required in Tarragona. At both sites, blooms are not related to conventional nutrients (nitrate, phosphate).

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First report on the bloom of toxic dinoflagellate *Alexandrium ostenfeldii* in Japan

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Alexandrium ostenfeldii has been identified as a toxic dinoflagellate producing paralytic shellfish toxins (PSTs) and/or cyclic imines (CIs). There are a few reports about the distribution of this species in Japan, but the bloom and the shellfish poisoning of the species has not been previously reported. However, in October 2013, we found the dominant species of the unidentified bloom in San-in district (western part of Honshu Island) as *A. ostenfeldii* through the morphological and phylogenetical analysis. Over 300,000 cells/L of *A. ostenfeldii* was observed by microscopy and neosaxitoxin and saxitoxin were detected by HPLC analysis in the residue obtained from the filtration (15 µm) of the water sample. Mouse bioassay (MBA) of the edible fresh water clam *Corbicula japonica* collected in the same water gave a positive result (2.1 MU/g) and the result in the MBA coincided with the HPLC analysis. Meanwhile CIs were not detected by LC/MS/MS analysis both in the water and the clam sample. The fresh water clam is important resource and the recurrence of the bloom is deeply concerned. Considering the high concentrations of cysts in the sediment samples (1,119 to 9,593 cysts/cm^3), we should continue to pay attention to *A. ostenfeldii* in brackish waters as the causative organism of PST in Japan.

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Gymnodimine and spirolide in shellfish during DSP toxicity in Central and Southern Adriatic Sea

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In the middle and southern Adriatic Sea the shellfish farms (*Mytilus galloprovincialis*) and harvesting area of the natural populations of warty venus (*Venus verrucosa*) are located. The contamination of shellfish with marine biotoxins is a food safety and human health concern. During 2011, the period of DSP (diarrhetic shellfish poisoning) toxicity was recorded, within a national monitoring program by the official analysis of marine biotoxins. DSP shellfish toxicity was revealed by mouse bioassay as official methods for DSP toxins. In some areas, the occurrence of toxicity started in January 2011 and lasted until mid-summer, with a maximum number of DSP positive samples during May and June. All samples, DSP positive and negative, were analysed by LC-MS/MS method. The most of the DSP positive samples contained gymnodimine and spirolide in low concentrations. We determined the levels and seasonal distribution of the gimnodimine and spirolide in the middle and southern Adriatic Sea. Gymnodimine and spirolide are already recognized as highly toxic by injection, as well as, fast-acting because of the rapid onset of neurological disorders and short survival time.

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Species Diversity and Biogeographical Distribution of *Pseudo-nitzschia* in Southeast China Coastal Waters

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Pseudo-nitzshcia is a common and worldwide distributed harmful diatom genus and some species have been reported to produce amnesic shellfish poisoning (ASP) toxin. Although the existence of toxic strains has not yet been reported in China, *Pseudo-nitzschia* species is widely distributed in Southeast China coastal waters. However, studies on species diversity and geographical distribution of *Pseudo-nitzschia* in China are very limited to individual local areas. In the present paper, we attempt to combine our investigation data and other published data to give a relatively complete description on the species diversity and biogeographical distribution of *Pseudo-nitzschia* in Southeast China coastal waters. In total, nineteen species of *Pseudo-nitzschia* have been recorded from Southeast China coastal waters: *P. americana*, *P. australis*, *P. brasiliana*, *P. caciantha*, *P. calliantha*, *P. cuspidata*, *P. delicatissima*, *P. heimii*, *P. mannii*, *P. micropora, P. multiseries*, *P. mutistriata*, *P. linea*, *P. pseudodelicatissima*, *P. pungens*, *P. seriata, P. sinica*, *P. subfraudulenta* and *P. subpacifica*. The species are recorded mainly from the six water areas: Changjiang River estuary, Taiwan Strait, Xiamen, Guangdong Province, Hong Kong, and Hainan. Among which, *P. pungens* and *P. pseudodelicatissima* are most widely distributed in the six water areas; *P. brasiliana* has been observed in warmer waters including Xiamen, Guangdong, Hongkong and Hainan; *P. cuspidate, P. calliantha* and *P. micropora* are available only in tropical waters. The species diversity (based on updated reports) are also different in these six water areas which is highest in Hongkong and Guangdong Province with 14 *Pseudo-nitzschia* species and which is relatively lower in other four areas with 5 species in Hainan and Changjiang River Estuary, 3 species in Xiamen and 2 species in Taiwan strait.
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Ship ballast arriving to Canadian ports as a dispersal vector for the diatom genus *Pseudo-nitzschia*

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Most harmful diatoms belong to the marine, planktonic genus *Pseudo-nitzschia* and are responsible for potentially lethal amnesic shellfish poisoning through production of domoic acid. Fifteen *Pseudo-nitzschia* species, ten of them toxigenic (approximately 30% of the genus) were recovered from 195 ship ballast tanks (water and sediment) destined for Canadian ports. Our results demonstrate that the three Canadian coastal regions receive considerable annual propagule pressure (1.2×10^{13}, 2.6×10^{13}, 1.5×10^{10} cells from ballast; 4.5×10^{9}, 3.7×10^{11}, 5.3×10^{6} cells from sediments), and colonization pressure (15, 11 and 3 species) from these diatoms for Atlantic, Pacific and Great Lakes ports, respectively. The Canadian Atlantic coast is under particularly high pressure from *P. turgidula*, and the Canadian Pacific from *P. seriata*. Both species are toxigenic and not yet reported from either region. Highly toxigenic *P. australis* has been recently found in Scottish waters, but not yet in Atlantic Canada. A greater number of species may be dispersed by ballast waters than by sediments because lightly silicified and narrow-valve species were absent in our sediment samples. However, survivors in the ballast sediments may be better adapted to tolerate suboptimal growth conditions when introduced to non-native regions/environments. Toxigenic *P. delicatissima*, found in Great Lakes ship ballast waters, is known for its tolerance of brackish waters and may be one of the coastal species with potential for establishment in the Great Lakes.

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Spatiotemporal Variability of Phytoplankton Blooms in Response to Nutrient Enrichment in Manila Bay

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The spatiotemporal trends of phytoplankton blooms in response to anthropogenic nutrient enrichment were studied in Manila Bay. We present here the species composition and distribution of phytoplankton from 2012 to 2013, and describe the nutrient inputs, & physico-chemical parameters of the Bay. A variety of 56~43 phytoplankton taxa comprising three major groups: diatoms, dinoflagellates and cyanobacteria were identified. Bacillariophyceae formed the most dominant group of phytoplankton in the Bay during dry and wet seasons with different wind monsoonal shifts for each year. Phytoplankton abundances in 2013 was significantly higher than in 2012. Previously recorded toxic dinoflagellates *Pyrodinium bahamense* var. *compressum* and *Gymnodinium catenatum* were not present in all sampling period. However, persistent blooms of a non-toxic dinoflagellate green and red *Noctiluca scintillans* has taken over the entire bay which were intermittently observed in May-August 2012 and January-March 2013. This study registered a number of harmful algae, potential threat for future bloom occurrences with risks of phycotoxins contamination due to increased eutrophication and climate change.

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Mesodinium rubrum red tides in Korean coastal waters – last 30 years

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Mesodinium rubrum is a cosmopolitan red tide ciliate and also an obligate mixotroph requiring cryptophycean prey to sustain photosynthesis and growth. Bloom formation by *M. rubrum* has been recorded in Korea since early 1980s. As part of these records, 40 cases of *M. rubrum* outbreaks in Korean coastal waters during last three decades were archived in Red Tide Monitoring Dataset of the National Fisheries Research and Development Institute of Korea. Distribution and ecology of Korean *M. rubrum* populations were first reported in an article about the red tide occurrence and species succession in Jinhae Bay (Park et al. 1988). The first ever temperate strain of *M. rubrum* was established from Gomso Bay, Korea in 2002 (Yih et al. 2004a), which was followed by a series of research on the biological and ecophysiological characteristics of the ciliate strain MR-MAL01 and then by the first successful cultivation of a DSP dinoflagellate, *Dinophysis acuminata* strain DA-MAL01 in 2006. Here, we summarize occurrence of *M. rubrum* in Korean coastal waters encompassing from the national wide long-term monitoring program to the spatiotemporally fine-scaled regional studies. In parallel, we review previous ecophysiological researches using Korean *M. rubrum* cultures and finally added our perspectives on the future directions of *M. rubrum* research in Korea.

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Nutrient contribution of submarine groundwater discharge in Harmful Algal Bloom–infested Sorsogon Bay, Luzon Island, Philippines

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Sorsogon Bay, an economically important fishing ground in the Philippines, has experienced toxic algal blooms from 2007 to 2011. However, nutrient loading – which has been linked with the promotion and persistence of harmful algal blooms (HABs) – into the bay were found to be within regulatory limits as revealed by a series of river samplings. As such, submarine groundwater discharge (SGD) was suggested as another source of nutrients into the bay. In this study, potential nutrient contributions of SGD were determined in one segment of the bay and were compared with riverine nutrient fluxes. A total of twelve Lee-type manual seepage meters were laid out in four 150-meter transects located southeast of the bay. Seepage was collected periodically for 36 continuous hours, of which nutrient samples were also taken for the first 24 hours. Integrated seepage flux of the transects ranged from 10.8 to 25.0 $Lmin^{-1}m^{-1}$ with an average of 17.0 $Lmin^{-1}m^{-1}$. Seepage waters have significantly higher nutrient concentrations than the ambient nearshore seawater by ~3 and ~4 times for dissolved silicate and phosphate, respectively. Extrapolating to the 100-km coastline of Sorsogon Bay, dissolved silicate and phosphate fluxes by SGD are 37% and 265%, respectively, of the nutrient fluxes of sampled rivers. Lower SGD silicate concentration was due to the elevation of riverine concentration by Mt. Bulusan's eruption in 2011. Higher SGD phosphate flux might have played an important role in bloom dynamics prior to the eruption as high P:Si ratio favor a flagellate-dominated community.

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Life cycle in *Pseudo-nitzschia* spp.: species-specific control of the induction of sexual reproduction.

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In toxic diatoms such as *Pseudo-nitzschia*, life cycle may influence population dynamics and toxin production. Better understanding of *Pseudo-nitzschia* sexual reproduction is thus crucial. The life cycle of three *Pseudo-nitzschia* species from the English Channel (*P. pungens*, *P. fraudulenta* and *P. delicatissima*) was studied in cultures. Mating experiments were carried out under three light intensities (30, 100 and 300 µmol photons m^2 s^{-1}) and a temperature gradient ranging from 8 to 26°C. The influence of light and temperature on the induction and timing of sexual reproduction was different among *Pseudo-nitzschia* species. Sexual stages were observed between 8 and 20°C in *P. fraudulenta* and *P. pungens*, but only below 16°C in *P. delicatissima*. The onset of the sexual phase occurred earlier under higher temperatures and higher irradiances. The fluorochrome PDMPO which labels the formation of biogenic silica structures was used to study the formation of auxospores and initial cell valves. Furthermore, the existence of intraspecific mating barriers amongst populations of *Pseudo-nitzschia* species of different geographic origin was tested. Sexual compatibility between *P. pungens* strains (clade I) from the English Channel and the Atlantic Ocean was observed. These results bring new understanding on the factors controlling the occurrence of sexual reproduction in *Pseudo-nitzschia* spp.

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Understanding the factors governing *Azadinium* generated shellfish toxicity in Scottish waters

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Azadinium is a newly characterised genus of dinoflagellate which has been shown capable of producing a potent biotoxin, azaspiracid (AZA). AZA was discovered after a poisoning event in the Netherlands in 1995 caused by mussels cultured in Ireland. Ireland has since had problems with AZAs in shellfish; however the type species *A. spinosum* was isolated from Scottish waters. Since Scotland's coastal currents are closely linked with that of Ireland the Scottish government are taking measures to understand more about the potential threat and begin regulatory monitoring. Toxin in shellfish flesh is already regulated by the competent authority (Food Standards Agency) but it would be ideal if cell numbers for *Azadinium* could also be monitored to allow better early warning.
Monitoring cells of *Azadinium* directly is problematic as they are small and look similar to many other species, in particular *Heterocapsa sp.*, under microscope observation. Therefore molecular techniques have been developed to monitor this species in environmental samples. Qualitative Polymerase Chain Reaction (qPCR) and CAtalysed Reporter Deposition Fluorescence In-Situ Hybridization (CARD-FISH) allow per cell determination of *Azadinium*. At the time of writing there is no available data on the link between environmental spread and abiotic variables which provide useful information for the design of monitoring programmes.
To remedy this we are conducting a year-long survey in several Scottish inshore locations to compare the molecular techniques developed for detecting *Azadinium*. We will use the distribution data to correlate environmental parameters to gain insight about abiotic factors in bloom development.

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Seasonal Dynamics of the Potentially Toxic and Harmful Phytoplankton in San Pedro Bay, Leyte, Philippines

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The seasonal dynamics of the abundance of potentially toxic and harmful marine phytoplankton in San Pedro Bay is elucidated for the first time. Three sampling stations were established and each were assessed for physico-chemical parameters in situ. All water samples were taken one meter below water surface for each station. For plankton samples, Vertical Alpha Water sampler (WILDCO) and a 20 µm plankton net with a length of 1 m and aperture diameter of 30 cm were used. Sampling was done for two years, in 2012 and 2013. A total of 18 potentially toxic and harmful phytoplankton were identified consisting of 1 cyanobacterium, 1 haptophyte, 1 diatom and 15 dinoflagellates. Seven of these reached significant densities within the sampling period thus their seasonal abundance were traced. There was a bloom of harmful cyanobacterium, *Trichodesmium* sp. during the month of April 2013 reaching 70,000 cells/L. Both *Phaeocystis* sp. and *Pseudo-nitzschia* sp. increased in abundance during the rainy season of both years particularly during the month of August 2012. Dinoflagellates also exhibited a relative increase in cell abundance during the rainy season of both years. High nutrient availability during the season must have influenced the behavior of both large groups despite differences in temperature, light intensity and DO concentrations among seasons.

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Differential okadaic acid accumulation by oysters and mussels under natural and simulated blooms of *Dinophysis acuminata* complex in southern Brazil

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A bloom of *D. acuminata* complex was detected in early March 2008 in the shellfish farming area of Baía Sul, Florianópolis (southern Brazil). Cell density reached up to $4.7x10^4$ cells.L^{-1} at the surface by March 31st, quickly decreasing (<$4.0x10^3$ cells.L^{-1}) 1-2 weeks later as salinity increased (>32) in all sampling stations. On average, commercial-sized mussels, *Perna perna*, accumulated 11-fold higher okadaic acid (OA) levels in the digestive glands (DG) compared to those of oysters, *Crassostrea gigas* (maximum 2422 and 271 ng.g^{-1}, respectively). Moreover, nearly all mussel DG extracts exhibited acute toxic effect as assessed by mouse bioassay, whereas oyster samples produced only negative results. In parallel, juvenile mussels (*P. perna*) and oysters (*Crassostrea brasiliana* and *C. gigas*) were exposed in the laboratory to a concentrated plankton suspension (20-60 µm) rich in *D. acuminata* complex cells (1350 to 13,750 cells.L^{-1}) for 24 h, followed by a 168-h depuration period on a non-toxic *Tetraselmis suecica* diet. OA was the only toxin detected in *Dinophysis* cells (1.7–3.3 pg.$cell^{-1}$), as well as in bivalve tissues, reaching 7.5, 23.4 and 51.1 ng.g^{-1} in whole bodies of *C. brasiliana*, *C. gigas* and *P. perna*, and 22.7, 66.1 and 183.3 ng.g^{-1} in their visceral tissues, respectively. Toxin levels dropped to similarly low average levels (3.6-3.7 ng.g^{-1}) in both oyster species and close to zero (1.3 ng g^{-1}) in mussels after 168 h. Mussels detoxified OA at faster rates (0.023 h^{-1}) than oysters *C. gigas* (0.010 h^{-1}) and *C. brasiliana* (0.004 h^{-1}), especially in the visceral tissues.

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Domoic acid production by *Pseudo-nizschia* spp. from southern Brazil, and feeding responses and toxin accumulation by exposed bivalves

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This study comprises a search for potentially toxic *Pseudo-nitzschia* species in southern Brazil; establishment of clonal cultures to evaluate their growth rates and capacity for domoic acid (DA) production; and quantification of feeding rates and DA accumulation in exposed bivalves. Fifteen out of 27 successful cultures were tested for toxin production, but only two produced DA (max. 0.054 fg.$cell^{-1}$ in *P. calliantha*; 0.154 pg.$cell^{-1}$ in *P. multiseries*). Additionally, *P. calliantha* was tested to infer whether constant light and/or turbulence would boost DA production. Although no toxin was detected, constant swirling sustained cell chain formation throughout the experiment, indicating that turbulence might affect *Pseudo-nitzschia* growth and life cycle in nature. When exposed to simulated unialgal *Pseudo-nitzschia* blooms, mussels *Perna perna* exhibited higher clearance rates than Pacific oysters *Crassostrea gigas* and mangrove oysters, *C. brasiliana* (4.1, 1.7 and 1.4 ml.min^{-1}.$individual^{-1}$, respectively). However, mangrove oysters *Crassostrea* sp. accumulated higher DA levels (up to 4.85 µg.g^{-1}) than mussels (<detection limit of 0.075 µg.g^{-1}) after short exposure to toxic *P. multiseries* cells (0.235 pgDA.$cell^{-1}$), suggesting low DA assimilation and/or rapid elimination by the latter. In *Crassostrea* sp., DA levels remained below the regulatory limit (20 µg.g^{-1}) over the 23-h toxin exposure period and the subsequent 168-h depuration period on a non-toxic diet, possibly associated with low feeding rates and/or selective *Pseudo-nitzschia* rejection. The low toxin content among the investigated *Pseudo-nitzschia* isolates, along with the low DA accumulation/ fast detoxification reported herein for these bivalves, suggest a low-risk scenario for ASP in this area under regular circumstances.

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Gymnodinium algae blooms impact on the recovery of the *Eriocheir sinensis* resource

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The life history of *Eriocheir sinensis* H. Milne-Edward has two periods: seawater stage and freshwater stage. In the Northern China, the period from reproductive stage to larval stage living in the seawater is about 7 months or so; the period from the development of larvae to sexual maturation stage living in the freshwater is about 17months of time. Widely used in China at present, the breeding of *Eriocheir sinensis* always uses hypaethral earthen ponds to cultivate the Megalopa larva as stock enhancement or seed resources for aquaculture production. Seedlings of *Eriocheir sinensis*, especially in the Zoaea period, is very sensitive to harmful algae such as *Gymnodinium*. *Gymnodinium* over a certain density in the water and becoming the dominant species can cause seedlings suffer from reduction in appetite and vigor, metamorphosis difficulty until death. It may accelerate water eutrophication, HAB are more likely to happen and cause mass mortality of seedlings when inputting amounts of rotifers and other exotic food in the high-density nursery pond. Although the aquatic breeding farm covers an area of about 6,700 hectares across the country, the areas of total loss or production cuts are more than 1,300 hectares and the loss of fry is up to 20% on account of harmful algae blooms. At present countermeasures, mainly replacing the subsoil of the pond before seedling production and removal of harmful algae's resting spore from the sources to prevent the occurrence of algae blooms.

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Geographical comparison in toxin profiles and genetic markers in the toxic diatom *Nitzschia navis-varingica* along the warm water current Kuroshio

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The benthic diatom *Nitzschia navis-varingica* is known to produce high levels of domoic acid, as well as a few isomers of domoic acid. Toxin profiles vary among strains. Previously *N. navis-varingica* has mainly been found in brackish tropical and subtropical areas in the Philippines, Vietnam, Thailand, Indonesia and Okinawa, Japan. It has, however, also been found in temperate brackish waters of the main land of Japan, but restricted to sun-exposed sites. The distribution sites were a little warmer than around. The distribution pattern in relation to the direction of the warm water current Kuroshio, running from the east coast of the Philippines to Japan, led to the hypothesis that *N. navis-varingica* is tropical in origin and that the warm current contribute to its dispersal. To test this hypothesis, key areas at the west and south coasts of Japan and Taiwan were sampled and cultures established. Culture experiments showed that *N. navis-varingica* survives wide salinity ranges (7–35) with the maximum growth at salinities between 21 and 28. Growth of *N. navis-varingica* at a salinity of 35, indicates that it may survive moving along the high salinity warm water current from tropical areas to Japan. Comparisons of the toxin profile types and analyses of ITS regions of rDNA of the strains shows similarities among the strains isolated from tropical starting areas, key areas and arrival areas along warm current Kuroshio, that may support the spreading hypothesis.

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Danube delta biosphere reserve (Romania) – a potential source of HABs?

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Although the incidence of HABs has special importance in geographical areas with high temperature, this phenomenon has been reported even in temperate areas, such as Romania. In the last 30 years, strong eutrophication was manifested in Danube Delta, especially in the context of climate change. The development of potentially toxic algal species appeared as an effect of the long-term affected ecosystems and had a significant impact on the entire food web. During 2013, an extensive study in 26 different lakes was initiated. The assessment of phytoplankton assemblage was based on *in situ* spectral fluorometric analyses, followed by microscopic assessment (taxonomic composition and specific abundance). Also, molecular methods were applied in order to detect the presence of responsible genes for toxin production. Species with toxic potential belonging to: *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Oscillatoria, Phormidium* and *Microcystis* genera were reported in all the studied lakes. Our purpose was to detect if these species have genes that synthesize one of the main types of cyanotoxins (anatoxin-a, microcystin or saxitoxin) and\or if these are responsible for the low level of zooplankton abundance found previously in studied lakes. According to our results, Cyanobacteria and their abundance influenced positively zooplankton main groups like: testaceans, rotifers or cladocerans. They also present a direct and negative correlation with Bacilarophyceae which betrays a competition between these two groups. In most of the lakes, preliminary tests revealed the presence of microcystin and saxitoxin genes. Further research will be done in 2014 as well, in order to detect more accurately the toxins types and their effect for the entire ecosystem and to help sustainable management of this valuable natural resource like Danube Delta Biosphere Reserve. *This work was supported by the Swiss Enlargement Contribution, project IZERZ0 – 142165, "CyanoArchive", in the framework of the Romanian-Swiss Research Programme.*

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Does phytoplankton community composition during drought periods favor *Karenia brevis* bloom development in the Mission-Aransas Estuary (Texas, USA)?

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Estuaries are particularly vulnerable to climate variability. Droughts are a common feature in Texas, where precipitation is highly variable among years. During periods of drought, low river discharges in estuaries are associated with an increase in salinity and a reduction of nutrients, which can cause alterations in the phytoplankton community structure. In the present study, we used the phytoplankton time series from the Imaging FlowCytoBot (IFCB) deployed at the entrance of the Mission-Aransas Estuary to investigate the community composition during and after drought conditions. In years 2009 and 2011, two prolonged drought periods occurred, which resulted in extended periods (3–4 months) of high salinity during summer. Overall phytoplankton abundance decreased during these periods, most evident in 2011, and very few species were present. When river discharges increased (September–October) due to precipitation, *Karenia brevis* cells were detected above background by the IFCB. It appeared the *K. brevis* cells originated offshore, and reached peak abundances of ~1400 and ~1700 cells/ml in 2009 and 2011, respectively. *K. brevis* represented from 30 to 60% of the total phytoplankton community during the development of the blooms. Therefore, the blooms coincided with increased river discharges in the estuary and a low dominance of the co-occurring phytoplankton species. One possibility is that conditions in the estuary could be more favorable to *K. brevis* due to suitable salinity and to the low abundance of the typical phytoplankton community found in the estuary as a consequence of the previous drought.

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Effect of allelopathy on the competition and succession of *Skeletonema costatum* and *Prorocentrum donghaiense*

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Prorocentrum donghaiense and *Skeletonema costatum* are typical phytoplankton species found in the East China Sea. These species coexist in natural phytoplankton communities. *P. donghaiense* blooms usually occur after *S. costatum* blooms in spring and summer. Although researchers have analyzed the relationship between these two species, the mechanisms controlling the succession of blooms remain unclear. The allelopathic chemicals produced by phytoplankton species are essential to the development and evolution of phytoplankton communities in a marine environment. A series of bi-algal cultures in seawater and *S. costatum* filtrate was conducted to examine the effect of allelopathy on the competition between *S. costatum* and *P. donghaiense*. The results indicated that *S. costatum* was the dominant species in the culture of seawater when nutrient concentrations and ratios in the cultures were similar to those in the field. The active bacteria were not the predominant factor affecting the competitive results. However, the evident succession between *S. costatum* and *P. donghaiense* in the *S. costatum* cell-free filtrate culture as the same nutrients conditions as the seawater cultures indicated that allelopathy had important effects on the competition and succession of *S. costatum* and *P. donghaiense*. This study provided insights into the succession phenomenon in the natural red tides occurring in the East China Sea.

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Dinophysis populations in the Celtic Sea, south of Ireland: Origin, transport and impacts.

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Approximately half of the economic losses caused by contamination of farmed shellfish with algal biotoxins within Ireland derive from the DSP suite of toxins produced by the dinoflagellate *Dinophysis.* These toxic events are always caused by the transport of toxin-producing populations from coastal waters into sites of shellfish aquaculture. The origin of these populations, characterised by a high survival capacity, has however remained obscure. Field results taken in early summer 2013 clearly show that in the Northern Celtic Sea *Dinophysis* populations first develop in an area known as the Nymphe Bank. They are then transported in the coastal current and impact when the current is forced onto the coastline. Monitoring data suggests that this population was transported west towards the bays of southwest Ireland which are used for intensive mussel culture, where it arrived approximately two weeks after its initial observation near the Nymphe Bank.
The results show that harmful *Dinophysis* populations can be transported from up to 150 km away from their point of impact., which has implications for their monitoring, prediction and management.

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Effects of plastid debris extracted from *Mesodinium rubrum* and cryptophyte cells on the growth and toxin production of *Dinophysis acuminata*

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As a causative species of diarrhetic shellfish poisoning (DSP), *Dinophysis acuminata* is widely studied on its ecological and physical characteristics. Influences of different environmental factors, including temperature, illumination and dissoloved inorganic nutrient, on this genus have been revealed. As a mixotrophic species, *Dinophysis* requires both light and prey to maintain its metabolism. Food type and quantity was proved to be the key factor on the growth and toxin production of *Dinophysis*. However, whether the *Dinophysis* could take up the organic matters extracted from its food prey are still ambiguous. Here, we investigate the research on growth and DSP toxin production of *D.acuminata* under different prey status: living *Mesodinium rubrum*, cell debris of living *M. rubrum*, living cryptophytes and cell debris of living cryptophytes. The cell debris of *M.rubrum* and cryptophytes was accomplished by ultrasonic processor and plasid debris (phycoerythrin autofluorescence) was photographed and measured using a Zeiss Axio Imager A1 microscope equipped with epifluorescence coupled to a Zeiss Axiocam MRc digital camera. Our preliminary experiment showed the positive growth of *Dinophysis* under cell debris of living *M.rubrum* condition, suggesting that plasid debris of *M.rubrum* could possible take an important role on the metabolism of *Dinophysis*. If so, how about the plastid debris from cryptophytes and/or other organisms which contain similar plasid? Therefore, our follow–up trials is to investigate 1) How the plasid debris from *M.rubrum*/cryptophytes affects the growth and toxin production of *Dinophysis*; 2) What plasid fraction takes the role? 3) Whether the debris from other organisms could be used by *Dinophysis*. After this research, we hope to answer the question that how would the *Dinophysis* survive when the *M.rubrum* are not available in the water and whether the distribution of certain type of cell debris plays a role of the mechanism of *Dinophysis* blooms.

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Environmental control of interannual variability in *Pseudo-nitzschia* species diversity and particulate domoic acid concentrations in the Bay of Seine (France)

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Some *Pseudo-nitzschia* species are domoic acid (DA) producers and are therefore responsible for Amnesic Shellfish Poisonning (ASP). In France, several ASP events occurred in the Bay of Seine since 2004. The dynamics of *Pseudo-nitzschia* spp. populations and the temporal evolution of particulate DA (pDA) concentrations were followed during two years, 2012 and 2013. While *Pseudo-nitzschia* spp. blooms occurred each year in spring and autumn, *Pseudo-nitzschia* species diversity and particulate domoic acid concentrations varied greatly between both years. In 2012, three different species were identified during the spring bloom (*P. australis*, *P. pungens* and *P. fraudulenta*) with high pDA concentrations (4070 $ng \cdot L^{-1}$) resulting in an ASP event. In contrast, the 2013 spring was characterized by a *P. delicatissima* bloom and no ASP event. The 2012 toxic *Pseudo-nitzschia* bloom was observed in conditions of potential silicate limitation (Si:N<1), when nitrate concentrations were still replete. Above all, the results from the two studied years show that high pDA concentrations coincided with the presence of *P. australis*. The contrasting climate conditions between 2012 and 2013 highlight different environmental controls that might favour the development either of *P. delicatissima* or *P. australis.*

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Influence of monsoon on the occurrence of phytoplankton blooms in a tropical bay

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Estuaries located along the west-coast of India are a unique tropical habitat influenced by distinctly higher runoff during south-west monsoon (June–September) as compared to non-monsoon (October–May) season. This phenomenon brings in characteristic seasonal changes in the abiotic and biotic factors. The phytoplankton blooms, which are heterogenous in form and function, are influenced by such environmental changes. In this study phytoplankton bloom dynamics was observed by quantifying live phytoplankton and chlorophyll at a fixed station (Dona Paula Bay, west-coast of India) every day from May 2008 – May 2010. Results pointed out that the occurrences of blooms are more during monsoon followed by post-monsoon (October–January) and pre-monsoon (February–May). Diatoms followed by dinoflagellates dominated the phytoplankton community. Blooms of diatoms such as *Asterionellopsis, Bacteriastrum, Chaetoceros, Ditylum, Fragilariopsis, Leptocylindrus, Pseudonitzschia, Skeletonema* and *Thalassionema* were observed. Irrespective of the season, bloom duration ranged between 1-6 days indicating that the blooms are wide spread and can significantly influence the systems metabolic balance. Depth of light penetration (>50cm–diatoms; >100cm–dinoflagellates), salinity (>20) and nutrient enrichment (especially nitrate) are the most important factors for the bloom formation in the region. Termination of blooms coincided with nitrate exhaustion. We conclude that the occurrence of several blooms due to variations in river runoff during monsoon as compared to non-monsoon seasons can be one of the distinguishing feature of a monsoonal estuary.

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Inhibition effects of aqueous extract from *Eucalyptus tereticornis* on growth of *Prorocentrum donghaiense*

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In previous studies, *Eucalyptus* were found to be highly phytotoxic towards terrestrial plants. *Prorocentrum donghaiense Lu* (*P. donghaiense*), a typical harmful algae in Asia, commonly blooms in the East China Sea, Japan, and South Korea Sea. This research aim to explore the cytotoxic effect of aqueous extracts from different parts of *Eucalyptus tereticornis Smith* (namely trunk, branches, leaves and roots) against the noxious algae *P. donghaiense* and its physiological attributes. While the aqueous extracts ($0.5g{\cdot}L^{-1}$) of trunks, branches, and roots of *E. tereticornis* showed little effects ($P>0.05$) on the growth of *P. donghaiense*, the aqueous extract of leaves significantly inhibited ($P<0.05$) the growth of tested algae, and the inhibitory rates (IR) raised with increased concentration from 0.05 to $0.5g{\cdot}L^{-1}$. After 96 h cultivation, four chlorophyll fluorescence parameters were unable to be significantly influenced by aqueous leaf extracts below $0.25g{\cdot}L^{-1}$ ($0.25g{\cdot}L^{-1}$ is included), whereas they were significantly affected at $0.5g{\cdot}L^{-1}$. The fluorescence yield tends to decrease as the concentration of leaves extract increased. Yet the activities of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), and the content of MDA in *P. donghaiense* increased. Optical microscope showed that the extracts severely damaged the cell membranes of microalgae and the microalgae cells were expanded and ruptured. It concluded that the aqueous extract of *E. tereticornis* leaves possess algal-suppressing ability and could be used as a potential tool for future *P. donghaiense* bloom management.

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Species of *Pseudo-nitzschia* on the Mexican Pacific coast associated to environmental conditions (1998-2010).

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Because of the potential toxicity of domoic acid of *Pseudo-nitzschia* blooms this study was initiated with the purpose of tracing the occurrence of this genus along the Mexican Pacific. Results revealed the presence of eight species. The study material consisted of 74 selected samples (net hauls and water samples) collected along 27 locations from 1998 through 2010. Light microscope observations on water mounts and examination of acid-cleaned material mounted in Naphrax provided information on the pre-identification of species. Scanning and Transmission Electron Microscopes were used to confirm the identification of species. The ultrastructure of all species is illustrated. Quantitative data using the inverted microscope showed that *P. australis* Frenguelli form occasional blooms along the west coast of Lower California being dominant, while *P. Pungens* (Grunow *ex* Cleve) Hasle, *P. pseudodelicatissima* (Hasle) Hasle and *P. subfraudulenta* (Hasle) Hasle, were widely distributed along the study area forming blooms during winter and spring-summer months. The species *P. pungens, P. pseudodelicatissima, P. australis* and *P. subfraudulenta* were found in great number in net samples. *P. calliantha* Lundholm, Moestrup & Hasle, *P lineola* (Cleve) Hasle, *P.* cf. *subpacifica* (Hasle) Hasle, *P. cf. heimii* Manguin were found in few numbers. Species distribution, relative abundance and morphologic data when available are given. Obtained results, review of the published records and satellite images suggest that blooms of *Pseudo-nitzschia* species are associated with cold fronts from the northern California Current.

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The occurrence of some *Pseudo-nitzschia* species in the southern Gulf of Mexico: Data Base 1979-2013

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A long-term oceanographic program in the southern Gulf of Mexico provided an opportunity to study the distribution of 10 *Pseudo-nitzschia* species known or presumed toxic species in the southern Gulf of Mexico. Material for this study consists of the analyses of selected data, net and water samples of phytoplankton from a data base of 23 oceanographic cruises conducted from 1979 to 2013. Light microscope (LM) observations on water mounts and examination of acid-cleaned material mounted in Naphrax provided information on the pre-identification species. With the use of both transmission and scanning electron microscopes most LM identifications were checked though. Results revealed the presence of *P. fraudulenta, P. delicatissima, P. multiseries, P. pseudodelicatissima, P. pungens, P. subfraudulenta, P. cf. subpacifica, P. lineola*, *P. subcurvata* and *P. cf seriata*. The highest values of relative abundance of the most frequent species were: *P. pseudodelicatissima* (729358 cells L^{-1}), *P. pungens* (181483 cells L^{-1}), *P. cf. subcurvata* (3055 cells L^{-1}) and *P. subfraudulenta* (184908 cells L^{-1}). The species appears widely distributed in the area of study. Morphometric data are given for each species. The distribution of some species is discussed. Ultrastructure by electron microscopes of all species is illustrated.

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Pseudo-nitzschia blooms, scallops toxicity and environmental context links along the French coastal waters

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The genus *Pseudo-nitzschia* is a cosmopolitan diatom that has been linked to many toxic events worldwide. King scallops' contamination by domoic acid is highly problematic because of its long time retention in this bivalve tissue, leading to long time fishery closures. Since 2004, first year of scallops' contamination, toxic events have become more frequent along the Atlantic/English channel coasts. The aim of this study is to describe spatial and temporal variability of toxic events and *Pseudo-nitzschia* blooms in six contrasted bays scattered along the French coast and investigate the role of environmental factors driving this variability. Two south Brittany sites, Quiberon bay and Concarneau bay, show perennial contamination and high *Pseudo-nitzschia* abundances, the Brest bay and the Seine bay are irregularly affected, the Pertuis Breton exhibits only one major toxic event related to a strong *Pseudo-nitzschia* bloom, while the Saint Brieuc bay did not show any significant contamination neither high *Pseudo-nitzschia* abundance. While high *Pseudo-nitzschia* abundance seems to be a prerequisite to induce scallops toxicity, the link is however not obvious, As *Pseudo-nitzschia* is only determined at the genus level the lack of information regarding toxic species could partly explain the gap between shellfish contamination and bloom intensity as well as the lack of clear relationship between toxicity and environmental forcing.

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A survey on toxic diatoms *Pseudo-nitzschia multiseries* and *Nitzschia navis-varingica* after the 2011 Japanese tsunami

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Previously, *Pseudo-nitzschia multiseries* has been found in Ofunato Bay and Okirai Bay, Japan and identified as the source of weakly toxic scallops in Ofunato Bay. *Nitzschia navis-varingica* producing high levels of domoic acid (DA) and its isomers has been found in brackish water areas along the east coast of Japan. The tsunami in northern Japan in 2011 severely affected coastal areas. In order to evaluate the ASP potential in the areas after the disaster, monitoring of the diatoms and DA levels of plankton net samples and cultured scallops were performed. *P. multiseries* were monitored in above mentioned two bays. *N. navis-varingica* was monitored at the estuarine areas yearly during summer. Analyses of the plankton net samples for ASP toxins showed presence of DA (20-170 pg L^{-1} sea water (Ofunato Bay, September and October, 2013) and 8-31 pg L^{-1} sea water (Okirai Bay, June, September and October, 2013). Strains of *P. multiseries* were successfully isolated from one of the net samples and toxicity was confirmed in culture experiments. Fortunately, ASP toxins were not found in cultured scallops. *N. navis-varingica* seemed to re-appear gradually in the affected area, starting in the north. It was not observed at all in 2011. In 2012, it was observed in Otsuchi and Soma, and in 2013 in Ofunato, Hitach and Ichihara. The results indicate that that *N. navis-varingica* may have either re-appeared from survived cells from less affected estuaries or was transported into the area with the Tsugaru warm water current flowing from north to south along the north east coast of Japan. This warm current departs from the Kuroshio warm current (see Romero et al.) flowing from the east coast of the Philippines to Japan.

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Pitfalls in microcystin extraction and recovery from human blood serum

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Microcystins (MCs) contaminate water bodies due to cyanobacterial blooms all over the world leading to a public risk of intoxication. For monitoring of human exposure, sensitive screening methods are needed. ELISA has been demonstrated as a robust routine tool for MC quantification in environmental samples. However, some problems regularly occur during sample preparation leading to MC loss and therefore underestimation of the true concentration. The aim of the presented study was to assess the pitfalls of the MC-extraction method from human blood serum with more detail. For this, MCs (MC-LR, MC-YR, MC-RR, MC-LA, MC-LW, MC-LF and defined MC mixtures) were spiked into serum and quantified using the commercially available Adda-ELISA (Abraxis, Warminster, PA, USA) after standard extraction (methanol extraction with subsequent SPE). To detect the potential influence of sample storage and preparation materials different types of material such as glass, standard polypropylene and surface-treated polypropylene were compared. Loss of MC during preparation and storage is largely dependent on (i) the handling of the stored material, (ii) the 'surface' of the storage material and (iii) the hydrophobicity of the MCs.

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Seasonality, morphology and toxicity of *Pyrodinium bahamense* var. *compressum* in a coastal area of the Central Red Sea

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In the Pacific and Indo-pacific harmful algal blooms (HABs) or red tides are often caused by *Pyrodinium bahamense*, a euryhaline species mainly found in tropical and subtropical areas. *Pyrodinium* produces saxitoxin a potent neurotoxin responsible for paralytic shellfish poisoning toxin (PSTs) in humans. *Pyrodinium* has been recorded in the Red Sea since early in the 20th century, but no blooms had previously been reported in the region. In the present study we followed a persistent bloom of *Pyrodinium bahamense* in a Bay North of Jeddah (Central Red Sea). The bloom was first detected in November 2012 and subsequently studied from September 2013 to May 2014. During this period we made cell counts and measured biotic and abiotic factors such as temperature, salinity, nutrients, irradiance, and chlorophyll monthly. The bloom peaked in mid November reaching cell densities of 10^5 cells per L^{-1}. We present morphological features revealed in scanning electron microscopy of *Pyrodinium bahamense* var. *compressa* from the bloom. In addition to data of saxitoxin production, we also provide a comparative molecular analysis between the present strain of *Pyrodinium* and strains from other regions using the small and large ribosomal DNA sub units.

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First report of potential harmful diatom *Pseudo-nitzschia* species (Bacillariophyceae) in Singapore coastal waters

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Harmful algal blooms (HABs) are increasing and expanding globally. In Singapore, many toxic phytoplankton species such as *Karenia* sp. and *Pseudo-nitzschia* species have been observed in recent monitoring of HABs. The marine diatom *Pseudo-nitzschia* is a known bloom-forming phytoplankton species, which causes blooms in many coastal waters. *Pseudo-nitzschia* is a neurotoxin domoic acid producer, which has been associated with Amnesic Shellfish Poisoning (ASP). More than one-third of the known *Pseudo-nitzschia* species has been reported as weakly to strong toxic. To date, despite the high diversity of *Pseudo-nitzschia* found in South China Sea, no domoic acid contamination or potential toxic *Pseudo-nitzschia* species have been confirmed in Singapore waters. To investigate the diversity of potentially toxic *Pseudo-nitzschia* species, plankton hauls using plankton net were carried out at St. John's Island located at Singapore Strait. Plankton samples were treated with acid wash before observing them using scanning electron microscope and transmission electron microscope. In additional, analysis of nuclear-encoded partial large subunit ribosomal DNA and internal transcript spacer region were performed to support the morphological identification. Five species of *Pseudo-nitzschia*; *Pseudo-nitzschia brasiliana*, *P. multistriata*, *P. micropora*, *P. cuspidata* and *P. pungens* were confirmed. The present of *Pseudo-nitzschia* species were documented for the first time in Singapore and this warrant the need to include these species in HABs monitoring program.

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Long term water quality and phytoplankton dynamics in response to a reduction in point source nutrient delivery

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Monthly water quality and chlorophyll-a (Chl-a) samples were collected over 17 years (1997 to 2013) from a creek in a temperate southeast Australian estuary, the Hawkesbury River, to assess biological responses to nutrient inputs from two sewage treatment plant (STP) facilities. In 2003, the program was supplemented with phytoplankton cell counts. This long term monitoring initiative presented a rare opportunity to test hypotheses about nutrient resources and harmful algae. Prior to the upgrade there were persistently high levels of phytoplankton, frequent blooms and regular violation of harmful algal bloom (HAB) trigger levels set for the aquaculture industry. Nitrogen concentrations significantly declined after the STP upgrade and there was a corresponding decline in phytoplankton abundance (Chl-a), particularly in the austral summer ($p < 0.05$). Chlorophyll-a concentrations were strongly positively correlated with seasonal changes in temperature prior to nutrient reductions, but the relationship with temperature was no longer significant after the upgrade. Between 2004 and 2008, 24 HAB species out of 150 phytoplankton taxa (109 genera) were recorded but total cumulative species richness and the number of toxin producing species have declined. Here we use a multivariate statistical approach to evaluate whether nutrients and other environmental factors correlate with HAB species in a system that has undergone a significant reduction in nutrient inputs.

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Novel harmful blooms of a pennate diatom eliminate nutrients from the water column of the Ariake Sea

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In the Ariake Sea, Japan, the pennate diatom *Asteroplanus karianus* has formed massive blooms each winter since 2008. During the time of peak bloom, seaweeds of the genus *Pyropia*, which are cultivated to make nori products, are discolored due to a nutrient deficiency. As the discoloration phenomenon is thought to be caused by nutrient depletion in the environment, it is essential to understand the relationship between *A. karianus* blooms and depletion of nutrients. We investigated the nutrient dynamics associated with *A. karianus* blooms in the Ariake Sea and measured the uptake of nitrate and phosphate by an axenic strain of *A. karianus*. Dissolved inorganic nitrogen and phosphorus in the coastal waters were significantly lower in areas where *A. karianus* cells were proliferating ($r \leq -0.955$, $p < 0.01$), and these nutrients were severely depleted during the period of blooming. In the laboratory, we determined the maximum uptake rate and half-saturation constant of *A. karianus* cultures and thereby demonstrated that *A. karianus* blooms significantly deplete nutrients in the water column. The winter blooms presumably eliminate dissolved inorganic nitrogen and phosphorus from the water column and may indirectly cause the nutrient deficiency of *Pyropia*.

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The roles of calcium in the growth and colony formation of *Phaeocystis globosa*

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It's widely acknowledged that the success of *Phaeocystisis* can be mainly attributed to colony formation, although the environmental factors that drive colony formation are not well understood. We conducted experiments to investigate the influences of Ca^{2+} on the growth and colony formation of *Phaeocystis globosa*. The growth of solitary cells, colony concentrations and chlorophyll *a* concentration were all suppressed when *P. globosa* grew under calcium-free conditions, where only a few solitary cells and colonies were observed. In contrast, solitary cell abundances were more than 1.0×10^6 cells ml^{-1} in the presence of calcium regardless of calcium concentration. Higher calcium concentration was clearly effective in supporting the colony formation. Colony number of *P. globosa* with calcium concentration of 3 µM was only less than 100 colonies ml^{-1}, and increased rapidly to 1200 colonies ml^{-1} once calcium concentration increased to 18 µM. In addition, colony diameter of *P. globosa* grew in high calcium concentration was far higher than those in lower calcium concentration. Our results suggested that calcium plays key roles in building the mucilaginous matrix of colonies in *P. globosa*.

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Microphytoplankton and harmful species distribution and abundance in fjords of Southern Chile.

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Harmful species genera of *Alexandrium*, *Dinophysis*, *Protoceratium* and *Pseudo-nitzschia*, are present in Southern Chile, sometimes in bloom conditions or associated to toxic outbreaks. A time series from 2007 to 2013 is considered, to understand the occurrence and distribution of these species in Reloncaví fjord (42°40'S), Puyuhuapi fjord (44°40'S) and Union channel (52°00'S).
Diatoms contributed with >98% of total cell density and the highest value (22.6×106 cells l-1) was found during the Spring 2009 in Reloncaví fjord, with Skeletonema costatum, contributing from North to South with 90%, 39% and 28% of total density.
The harmful species are numerically subordinated with <1% of total number, the exception are *Pseudo-nitzschia cf pseudodelicatissima* and *P. cf. australis* contributing both with 45% of total density in Puyuhuapi fjord. Both species are present in low numbers in the other studied areas, 0.3%, Reloncaví fjord and 5.5%, Union channel. During the studied period, *Alexandrium catenella*, *A. ostenfeldii* and *Dinophysis acuta* in the Reloncaví fjord, were absent, while these species plus *D. acuminata* and *Protoceratiun reticulatum* were present in the other areas.
The microphytoplankton composition and distribution showed latitudinal changes associated with vertical stability and stratification of water column. The mean (±SD) of species diversity varied from 0.63±0.59 in Reloncavi fjord to 1.3±0.73 and 1.39±0.57 in Puyuhuapi fjord and Union channel, respectively.
Although the studied zones are geographic, oceanographic, and climatically different, the abundance of harmful species are regulated by climatic and oceanographic factors of wide geographic coverage and then, microphytoplankton distribution and abundance is locally driven.

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The recurrent and localized blooms of harmful dinoflagellate *Cochlodinium polykrikoides* in the southeast coastal waters of Korea

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The large endemic outbreaks of *Cochlodinium polykrikoides* in Korean coastal waters are a common phenomena and many sophisticated oceanographic studies had been conducted during last two decades. But all of these studies have been focused on offshore blooming events. The purpose of the present research was to consider some regional factors (i.e. resting cysts, seed beds, etc) that might have a great contribution to on-shore (i.e. Jaran Bay) blooming patterns, and to draw distinct blooming mechanism between on-shore and off-shore (i.e Mijo and Yeokji coasts). We first confirmed the existence of resting cysts that created local seed-beds responsible for recurrent blooming. The successful germination of resting and hyaline cyst had been observed in July–August and created blooms under favorable environmental conditions (i.e. temperature, salinity, etc.). The low resting cyst production also controlled the intensity of blooming pattern and responsible for less production of *C. polykrikoides* in Jaran Bay, although some unfavorable conditions had been detected during August 2011. In addition, the high abundances of *Nematodinium* cysts were also shown in water bodies where blooms occurred last year and that regions take the role of point sources of recurrent bloom initiation. Nevertheless, the off-shore blooming mechanisms are largely depending on physical oceanographic factors.

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Historical observations of Harmful Algal Blooms in two bays of the Mexican Pacific (1980- 2010).

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Daily color visual-observations and species composition of Banderas Bay (BB) and Mazatlan Bay (MB) have been carried out during the last 30 and 10 years respectively. Sampling procedures were obtained according UNESCO-IOC manuals. Results show that the discoloration days (dd) in the water are very variable, being the maximum (3.3 times more) in the 2000 decade in both bays. BB registered 579 discolored days and MB 604, although the number of blooms was smaller in BB with 57 blooms, while in MB 80 events were registered. Monthly relative phytoplankton abundance was similar too, with two peak values in spring and autumn suggesting that environmental conditions are similar. Events duration depends on species composition and hydrographic conditions. Thus, BB was characterized by blooms lasting from one week to a month, while in MB most blooms were ephemeral, lasting 1-2 days. The most frequent species in BB were: *Mesodinium rubrum*, *Cochlodinium polykrikoides* and *Leptocylindurs danicus*. The first species had ephemeral presence while the second represented harmful effects upon local ichthyo fauna of commercial importance. In BM, the most frequent species were: *Mesodinium rubrum*, *Gymnodinium catenatum* with toxic effects and *Skeletonema costatum*. Dominant species composition and environmental conditions are discussed. Satellite images when available were used to confirm some of these blooms.

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Monitoring of algal blooms along the southwest coast of India during 2008 to 2012

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Blooms of microalgae are very frequent in coastal waters around the world. As part of the harmful algal monitoring programme of the Ministry of Earth Sciences, Govt. of India, regular surveillance had been made along the Kerala coast, Southwest coast of India, one of the most productive areas in the world, during 2008 to 2012. This paper describes the observations made during the investigations. Four major bloom events were recorded during the study period. A bloom of *Protoperidinium* sp. was observed off Mangalore (12º 51.83'N & 74º 20.00'E) during an interdisciplinary cruise on board FORV Sagar Sampada. A mono-specific bloom of *Prymnesium parvum* N. Carter was observed off Azheekode (10° 11' 02"N & 76°09' 22"E) during the monsoon period (August 2009). A bloom of centric diatom *Proboscia alata* (Brightwell) Sandstrom was the third bloom event observed from 10th to 12th October 2009 along the coastal area near Bekalam (12° 24' 04"N & 75° 03' 03"E). A massive bloom of the marine raphidophyte, *Chattonella marina* (Subrahmanyan) Hara et Chihara was observed in the coastal sea off Mahe (11° 42' 18"N & 75° 32' 36"E) during October 27th to November 1st, 2011.Detailed investigations on the taxonomy, abundance and ecology of these microalgae have been carried out in order to understand the bloom dynamics.

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Harmful Algal Blooms in Singapore coastal waters

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Singapore coastal environments are increasingly affected by regular harmful algal blooms (HABs). The increase in frequencies of HABs have led to enhanced interest in monitoring and detecting of such blooms. In December 2009, a toxic bloom hit Singapore waters along the Johor Strait for the first time, causing massive fish kills and economic losses. Monitoring of HABs is essential to describe the trends of blooms, and thus providing a means for protecting aquacultures and public health. Our approach involves multi-scale-sensing using autonomous surface vehicles (ASV). In this study, we examined the conditions favorable for blooms to develop, reviewed past HABs and also a recent fish kill event. High spatial resolution data were collected using ASV, and distinct biological and physical patterns were observed. During the sampling period, a toxic dinoflagellate bloom was detected. Prior to the bloom, high concentrations of ammonium, nitrate, phosphate and silicate were observed. Apart from silicate, all other nutrients were found to be low in concentration after the bloom. We have detected three naked toxic dinoflagellates in our local waters not reported previously. Moreover, bloom was generally formed during neap tide and under favorable physical settings. In addition, the eutrophic conditions could be the driving force for bloom to develop along Johor Strait. The information observed could assist in defining bloom parameters and enhance our ability in determining and detecting pre-bloom condition. The platforms used in this study could assist in collecting high spatial resolution data set, which was not possible with point sampling.

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Inter- and intra- annual variations of harmful micro-phytoplankton from the Bay of Bengal

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The variation in the distribution and abundance of harmful micro-phytoplankton species at inter-and intra-annual scale was investigated along the shipping routes, viz Chennai-Port Blair (C-P transect) and Port Blair-Kolkata (P-K transect) in the Bay of Bengal. In this study, 46 harmful species of diatoms and dinoflagellates were recorded. Their abundance varied from 5 to 10 $\times 10^3$ cells L^{-1} along the CP transect and up to 31.9 $\times 10^3$ cells L^{-1}along the PK transect. High abundance of *Pseudo-nitzschia delicatissma* and *P. seriata* was noticed round the year and bloomed during the south-west monsoon, on two occasions in the P-K transect. These can be attributed towards freshwater discharge which brings relatively high nutrient concentration and low salinity. The other harmful dinoflagellate taxa such as *Scrippsiella trochoidea, Neoceratium furca, Neoceratium fusus, Prorocentrum micans, Amphidinium* spp. and *Gymnodinium* spp. are wide spread in its distribution in both C-P and P-K transects. The presence of these dinoflagellate species all-round the year indicates its survival under low nutrient condition through mixotrophic mode that can cope up with changing environmental settings. In addition to this, high abundance of *Dinophysis caudata* and *Dinophysis* spp. in the P-K transect can be linked to low saline condition. This study points out that *Pseudo-nitzschia delicatissma* and *P. seriata* can be considered as potential species for harmful algal bloom events.

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Relationship between occurrence of the toxic dinoflagellate *Dinophysis acuminata* and ciliate *Mesodinium rubrum* in the coastal water of Japan

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It is currently known that the toxic dinoflagellate, *D. acuminata* and other several *Dinophysis* species are successfully cultivated in the laboratory by feeding on a mixotrophic ciliate, *Mesodinium rubrum*. However, their prey–predator relationships are not well informed via *in situ* investigation. In this study, the occurrence of *D. acuminata* and *M. rubrum* was monitored in the coastal water of Japan. Water sampling has been conducted weekly or biweekly in an enclosed port located at Ise Bay, central Japan, from March 2012 to March 2014. The water samples were preserved with Bouin's solution and both organisms were enumerated under a microscope. *D. acuminata* occurred at > 100 cells L^{-1} from April to October 2012 and from April to September 2013, although the cell density was fluctuated widely. The maximum cell density, 7,590 cells L^{-1}, was recorded in June 2012. *M. rubrum* was always detected during the study period, although also fluctuated, in the range of 20 – 4,920 cells L^{-1}. The temporal changes in the abundances of both organisms often displayed the dynamics in which *D. acuminata* was flourished after the increase of *M. rubrum* and vice versa, indicating their prey–predator relationship in nature. Gene analyses in the food vacuoles of the natural cells of *Dinophysis* species were pioneered in this study. From the gene evidences, as prey items, *M. rubrum* was confirmed for *D. acuminata* and, furthermore, *Strombidium* sp., *Laboea* sp. and *Tintinnopsis* sp. as well as *M. rubrum* were firstly revealed for *D. rotundata*.

P-176 ST-13

Seasonal and spatial patterns in the abundance of potentially toxic species of *Dinophysis* and *Pseudo-nitzschia* in a subtropical Brazilian estuary

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This study investigates the spatial and temporal distribution of potentially toxic *Dinophysis* and *Pseudo-nitzschia* spp. in Guaratuba Bay, southern Brazil, a very dynamic estuary subjected to complex ecological and physical processes. The highest abundances of *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia pungens* (maximum 7.3 x 10^4 cells L^{-1}) were correlated with higher temperatures (from December to April, 22-30 °C), salinity (>20) and silicate concentrations (20-100 µM). Although high silicate concentrations stimulated the growth of these potentially harmful diatoms, cell abundances remained low to moderate as a result of growth limitation by phosphate and light in summer (rainy season). Moreover, the occurrence of *Pseudo-nitzschia* spp. in the bay probably depends upon the development of an inoculum population in the adjacent coastal water and its subsequent advection by tidal currents to the interior of the estuary. In addition, harmful species of *Dinophysis* were recorded in all monthly sampling campaigns performed between October 2010 and April 2012. The highest abundances of *Dinophysis acuminata*, *Dinophysis caudata* and *Dinophysis tripos* were found in the upper halocline layer, in regions of the bay where water column stratification was more frequent. On some occasions, cell density of *D. acuminata* (up to 3.2 x 10^3 cells L^{-1}) surpassed alarming levels in areas used as farming sites for bivalves. Abundance of *Dinophysis* spp. was not related to any variable investigated, but was rather dependent on the stratification of the water column.

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Trichodesmium blooms in south eastern Arabian Seas (SEAS): Significance of diatom-diazotrophic cyanobacterial associations in bloom event

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The present study was taken up with an objective of identifying the role of cyanobacteria and heterotrophic bacteria that are capable of fixing nitrogen, their distribution and diversity in the South Eastern Arabian Sea (SEAS: 9-13°N, 73-77°E). Sampling was carried out from the study area for a period of one year (March 2012 to February 2013) and analyzed for physico-chemical variables, nutrient profiles and distribution of nitrogen fixing heterotrophs and cyanobacteria. *Trichodesmium erythraeum* was found to be an important nitrogen fixing cyanobacteria in the SEAS forming blooms during spring inter monsoon and winter monsoon. *Trichodesmium erythraeum* bloom showed a density of 1.84 x 10^6 filaments/L during spring intermonsoon and 3.34 x 10^5 filaments/L during winter monsoon. Heterotrophic nitrogen fixing bacteria showed highest abundance in fall intermonsoon at northern part of south eastern Arabian Sea (3.8x10^4 to 7x10^4 cfu/ml) and lowest in winter monsoon (10 to 625 cfu/ml) in southern part of south eastern Arabian Sea. Heterocystous cyanobacteria such as *Calothrix* sp., non heterocystous cyanobacteria such as *Lyngbya* sp. and unicellular cyanobacteria such as *Synechocystis* sp. which are capable of carrying out nitrogen fixation were also encountered in the coastal waters of southeastern Arabian Sea. The study has also revealed frequent occurrence of diatom diazotrophic cyanobacterial associations in south eastern Arabian Sea. The results of the experiment showed that there was a functional N_2-fixing community in seawater and isolates of heterotrophic diazotrophs were obtained. Presence of *nif H* gene indicating N_2-fixing activity among the isolated heterotrophic bacteria were also detected.

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Variability in mesozooplankton community structure during an algal bloom in Bolinao-Anda, Pangasinan

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The Bolinao-Anda Channel is one of the areas where Harmful Algal Blooms occur every year. One possible cause of this is nutrient loading from the mariculture areas situated within the channel. Zooplankton play an important role in the energy transfer in the marine food web and may be carriers of toxins, which they obtain through feeding on Harmful Algal Bloom species and pass on to higher trophic levels. Zooplankton can also play an important role in regulating harmful algal blooms through their predatory activities. Mesozooplankton (0.2-200um) serve as grazers of primary producers and in return, are grazed upon by larger organisms including other zooplankton. These mesozooplankton are also involved in the transfer of organic matter throughout the water column through the re-suspension and sinking of such compounds. In order to understand the community structure of mesozooplankton during an algal bloom, sampling covering the peaks of the tidal cycle was conducted using a 280um plankton net in ten stations within the channel. Simultaneously, biomass of the dominant algal groups was determined using a multi-wavelength excitation fluorometer (Infinity Me). Zooplankton samples were fixed and brought to the laboratory for microscopic analysis. The effects of the tidal cycle and co-occurrence with algal groups were explored in order to help understand plankton dynamics during algal blooms. *Acartia* sp. was found to be dominant in all stations comprising approximately 94% of the samples. Also, high zooplankton biomass was observed for stations where high phytoplankton density was observed.

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The impact of the Taiwan Warm Current to coastal dinoflagellate blooms in the East China Sea

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An increasing trend in both frequency and intensity of dinoflagellate blooms occurred in the East China Sea (ECS). The foundational reason is the increasing nutrient concentration. In the ECS, a distinct feature is the Taiwan Warm Current (TWC) flowing northward across the wide continental shelf plain all the year round. Recently, we proposed that the large-scale algal bloom of the dinoflagellate *Prorocentrum donghaiense* developed from the population at the front of the TWC. And also both the *P. donghaiense* and *Karlodinium veneficum* blooms rapidly developed when the stratified water formed which was attributed to the movement of the TWC in 2011. Therefore, the TWC may contribute to those dinoflagellate blooms from seed bank, water column, nutrient, temperature and salinity and so on. Although impacts from overfishing, irregular pollution discharge, climate change and so on, should be taken into account, the impact of the TWC is direct and grateful. Such ocean currents which play multi-direct roles to dinoflagellate blooms in coastal marine systems deserve more attentions.

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IAEA activities to Enhance Developing Nations' Capacity for Management of Harmful Algae in the context of a changing world

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HAB toxins regularly affect international trade and sustainable coastal fisheries development through their accumulation in seafood product to levels that may adversely affect human health or through direct toxic effects on aquatic living organisms. The impacts are particularly significant in developing countries including the highly vulnerable small island developing states (SIDS). In partnership with international organizations the International Atomic Energy Agency (IAEA) is supporting technical cooperation (via 2 National and 3 regional TC projects on HABs and/or Ocean Acidification), applied research and method development using isotopic and radioisotopic approaches, to assist member states in managing marine resources and seafood safety, in particular to strengthen the capacity for prevention, management and mitigation of health and socio-economic impacts of HAB toxins.,

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Cross-platform performance assessment of colorimetric and microscopic detection of *Alexandrium fundyense/tamarense*.

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The *Alexandrium fundyense/tamarense* species complex is harmful algal bloom (HAB)-causing dinoflagellates that produce a suite of toxins responsible for paralytic shellfish poisoning (PSP). Spatial and temporal monitoring of the abundance of *Alexandrium* cells in the water is important, since even low cell densities can be toxic. However, current detection methods rely on microscopic cell counting, which is time-consuming and requires special training due to the almost-indistinguishable morphological similarities between toxic and non-toxic *Alexandrium*. We have developed a rapid and specific colorimetric assay using peptide nucleic acid (PNA) probes and the symmetric cyanine dye 3,3′-diethylthiadicarbocyanine iodide ($DiSC_2(5)$). The addition of $DiSC_2(5)$ to a solution with PNA-containing hybrids causes a color change from blue to purple. This change can be quantitatively monitored as an increase in the solution absorbance at 540 nm (from the dye aggregates) at the expense of the dye monomer peak at 650 nm, and the "hybridization signal" is calculated as the ratio A_{540}/A_{650}. A Portable Optical Sensing System for Environmental samples (POSSE) is a field-compatible two-wavelength colorimeter designed to quantify *Alexandrium* species cell numbers using the colorimetric assay that has been developed. In conjunction with the Maine State Department of Marine Resources phytoplankton monitoring program the POSSE instrument is assessed for sensitivity and specificity compared with standard microscopic methods during natural bloom and non-bloom conditions. The POSSE instrument is capable of detecting *Alexandrium* with high specificity, however this assay requires a larger volume of sample to reach appropriate sensitivity.

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Identification and tracking of Harmful Algal Blooms using multi-spectral techniques from remotely piloted aircraft platforms

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The use of remotely piloted aircrafts (RPAs) is potentially a novel means of remotely sensing phytoplankton and harmful algal blooms (HABs). RPAs make it possible to take measurements in areas which are otherwise difficult to access or are frequently obscured from satellites by cloud cover. The potential also exists for higher resolution imaging than is possible via alternative remote sensing techniques. This project will utilise a multi- or hyper-spectral camera mounted on an RPA, investigating the potential to remotely differentiate between phytoplankton and other dissolved matter within coastal waters. Subsequently, the project will investigate the multi-spectral differentiation of diatoms and dinoflagellates before examining specific species of HABs for distinctive characteristics.

Initially, a selection of nine harmful and benign species are being cultured for analysis of their optical properties, chlorophyll-a and cell counts. Using existing algorithms within the computer programs 'Hydrolight' and 'Ecolight', expected reflectance measurements are being generated for each. This will help to determine whether it is possible to remotely detect HABs by selecting for specific wavelengths of interest, either pre- or post-flight. Experiments are being repeated during different growth phases to investigate the impact of this on results. Further research will involve integrating a suitable spectral imaging camera into the existing RPA. Following test flights, surveys will be carried out during peak HAB season via opportunistic flights over HAB events, using SAMS' boats for launch and recovery of the fixed-wing RPA.

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Geographic Information System (GIS) maps for the temporal and spatial distribution of the toxic phytoplankton species in Kuwait Waters

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Seawater samples were collected during the year 2005 by Niskin sampler from different stations in Kuwait waters, A, B, 3, K6, 6, 7,and 18. Al-Bidaa station was sampled for plankton net samples (20 µm) in January, February and March 2010. Qualitative analysis of potentially harmful algal bloom species found in plankton net (20 um) samples for the year 2005 and the quantitative analysis of the toxic species found in the four selected seasons for the year 2005 were presented by Geographic Information System (GIS) maps. The maps show the temporal and spatial distribution during the year 2005. The toxic species found in the present study are the dinoflagellates, *Alexandrium minutum, Cochlodonium polykrikoides*, *Dinophysis caudata*, *Dinophysis miles*, *Dinophysis tripos*, *Gonyaulax spinifera*, *Gymnodinium catenatum*, *Karenia* cf. *Papilionacea*, *Lingulodinium polyedrum*, *Noctiluca scintillans*, *Phalacroma mitra*, *Phalacroma rotundata*, *Protoceratium reticulatum*, *Prorocentrum rhathymum*, *Pyrodinium bahamense* Plate 1906 var. *compressum*, the raphidophyte *Chattonella* sp., and the blue green algae *Trichodesmium erythraeum*. The dinoflagellate *Dinophysis caudata* shows the highest percentage of cell densities among the total phytoplankton in Kuwait waters in October 2005.

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A simple method for minimizing the harmful effect of phytoplankton blooms: the New Zealand King Salmon monitoring system on sea farms.

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Harmful phytoplankton blooms can cause devastating effects within the aquaculture industry. From first observable signs developing into a mass mortality event can transpire over a short time period with important financial consequences. New Zealand King Salmon (NZKS) is New Zealand's largest Chinook salmon (*Oncorhynchus tashawytscha*) producer. In 2010 one of NZKS marine farms experienced a catastrophic toxic algal bloom caused by the dictyochophyte *Pseudochattonella verruculosa*, resulting in approximately 200 tonnes of harvestable salmon dying over a six day period (MacKenzie et al., 2011). Traditionally, NZKS farm personnel would collect water samples on a weekly basis which was then couriered to Cawthron Institute for detailed analysis. Due to the fast and dramatic effects of harmful blooms as experienced by NZKS, an additional system was necessary for early detection and empowers a response from the farms to harmful toxic algae blooms. This new methodology is based on practical training, daily sampling and auditable record keeping. The new system has considerable advantages; it is performed daily or on demand using simple microscope techniques, affordable, effective through immediate response to potential harmful blooms and engages farm staff in understanding phytoplankton communities. We present here the methodology and systems employed by NZKS farm personnel. We demonstrate the link between the science fraternity and NZKS farm staff through support and training. Finally, we validate the necessity of specific training, staff engagement and timely continuous analysis of phytoplankton communities within the salmon farm microcosm.

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Risk-Monitoring, Modelling and Mitigation (M3-HABs) of benthic microalgal blooms across Mediterranean region.

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The pan-Mediterranean project M3-HABs regarding monitoring of harmful algal blooms, with particular reference to the benthic dinoflagellate *Ostreopsis* cf. *ovata* has recently started in the framework of the ENPI-CBCMED Programme. Along European Mediterranean coasts, *Ostreopsis* blooms have been so far reported in Italy, Spain, France, Croatia and Greece, and *Ostreopsis* occurrence has been recently reported also in other Mediterranean Countries, such as Tunisia, Egypt and Lebanon. The specific objective of the project is to provide a common pan-Mediterranean strategy for monitoring benthic toxic microalgae, through the development of new, more efficient and common procedures and protocols, making the process mostly cost and time effective, allowing for most efficient monitoring designs, increasing the knowledge on environmental drivers affecting *Ostreopsis* blooms and translate this into a forecasting tool, and improving the general awareness of the risks related to *Ostreopsis*. Given the specific objective of the project, the following results are expected: a larger awareness of the risks associated to the *Ostreopsis* blooms, an appropriate diffusion of caution measures set up, the production of common monitoring protocols, the development of new technologies for species-specific identification and counting and the build-up of prediction models in order to prevent and reduce risk factors for the environment, human health and economic activities. The project will improve the establishment of solid networks along Mediterranean coasts to cope with the *Ostreopsis* emergencies, providing the target groups, common and intercalibrated protocols, in order to have comparable samplings in space and time through the Mediterranean Sea.

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Harmful phenomena detected by the help of citizen participation

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PHENOMER, a citizen monitoring program of water discoloration observations and fauna mortality caused by phytoplankton proliferations has been launched across Brittany's (France) coastal waters in 2013. Beyond communication and out-reaches objectives, the project aims at exploring the possibility to acquire scientific valuable data on HABs extending the monitoring survey area of coastal water by means of citizen alerts. A theoretically infinite number of sampling points (public observations) will likely contribute to identify i) HABs frequency and recurrence; ii) distribution and extension of water discolorations; iii) biogeography of causative taxa. During the first year of project implementation 15 out of 40 observations corresponded to HAB events. *Noctiluca scintillans* red discolorations were detected at surface in July-August, at temperatures higher than 19°C, in correlation with western winds and variable tide coefficients. Almost synchronous observations were made at ca. 200 km of distance. Local *Lepidodinium chlorophorum* green discolorations were observed during a short period in August. One bivalve mortality event coincided with a dark-brown phytoplankton bloom characterized by the dominance of the toxic raphidophytes *Heterosigma akashiwo* and *Pseudochattonella verruculosa*. Interannual variations and recurrence of harmful bloom will be further evaluated thanks to new, future citizen observations.

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Shellfish monitoring for lipophilic phycotoxins in France: Recommendation for an updated sampling strategy

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The sampling strategy for the official monitoring of phycotoxins in bivalve shellfish in France differs according to the family of toxins and to the type of zone (coastal or offshore). For PSP and ASP toxins, the strategy relies on the monitoring of phytoplankton in seawater. The detection of toxic species above an alert threshold acts as a trigger for the analysis of toxins in shellfish. For lipophilic toxins, the phytoplankton is not a reliable indicator. A systematic weekly analysis of toxins in shellfish is performed in risk areas during high risk periods. Since 2010, high risk periods are defined as follows: the occurrence of one result above the European regulatory limit (160 µg equivalent okadaic acid/kg shellfish) over the last 3 years lead to consider the month as a high risk period. This definition has been recommended by the French food safety agency (ANSES) based on a statistical analysis of the official monitoring results for the period 2003-2008. At that time, the mouse bioassay (MBA) was the official analytical method but as of the 1st January 2010, it has been replaced by LC-MS/MS. By early 2014, ANSES initiated a new statistical analysis, based this time on results for the period 2010-2013 for which quantitative LC-MS/MS data are available (and not only qualitative positive/negative results from the MBA). We tested the robustness of the definition set in 2010 and identified a new methodology to improve our sampling strategy for lipophilic toxins in bivalve shellfish.

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Practical applications using diffusion coefficients derived from satellite imagery for studies of marine planktonic ecosystems

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The high-resolution satellite-derived images of 500 m spatial and hourly temporal resolutions are examined to quantify the diffusion coefficients of the sea surface chlorophyll concentration. Based on the advection-diffusion model, the optimal solution of the diffusion coefficients are estimated using advection terms associated with the tidal and geostrophic currents and the finite differences of concentrations in time and space. We implement the estimated diffusion coefficients in the random walk and flight models, which have been used for tracking of the concentration of phytoplankton, zooplankton, and individuals of fishes, and compare the satellite data and model outputs. This work will be applicable to the ecosystem process studies at submesoscale [*O*(1) km spatial scale and *O*(1) hour time scale] and improve the limitation of the present-day statistical modelling.

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The 2012 *Karlodinium* spp. bloom scenario in Swan River estuary

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A bloom of *Karlodinium* spp. was again observed in Swan River estuary in 2012 and caused massive fish kills. Although bloom of *Karlodinium veneficum* has dominated in the estuary since 2003, the bloom of this species in 2012 co-occurred with other *Karlodinium* cells, which varied in sizes. The bloom of *Karlodinium* spp. in Swan River estuary was captured during the regular weekly monitoring. A total of 12 regular sites that represents the lower, middle and upper sections of the estuary were monitored weekly for *Karlodinium* spp. abundance, and covers the various seasons (summer, autumn, winter and spring). Various physical (salinity, temperature, dissolved oxygen and pH) and chemical parameters (nutrients) were also measured and correlated with the species abundance. In this event, the bloom initiated in the upper section of the estuary in May (late autumn), middle and lower sections in June (early winter) with highest concentrations observed in the middle to upper sections. The spatio-temporal distribution of *Karlodinium* spp. during the bloom also showed a response to the environmental changes along the estuary. This study also highlights the need to use species-specific probes in the identification of *Karlodinium veneficum* in Swan River estuary during the routine monitoring to better understand the bloom dynamics of this species in response to various environmental parameters.

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The risk to New Zealand shellfish aquaculture from paralytic shellfish poisoning (PSP) toxins

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New Zealand's reputation as a supplier of high quality food products is vital to the national economy; international consumers are acutely aware of food safety issues and markets are increasingly demanding higher standards. Filter feeding bivalves are particularly sensitive to the nature of the environment in which they are grown, and quality assurance is a major preoccupation of the shellfish aquaculture industry. With exception of a couple of incidents, most notably the *Gymnodinium catenatum* blooms in 2000–2003, to date paralytic shellfish toxin (PST) contamination has not had an important effect on the economics and sustainability of the industry. However, the dinoflagellate species responsible for producing these toxins are common in NZ coastal phytoplankton communities, and it is important that awareness of the risk is maintained. This review summarises what we know about the causes and incidence of PST contamination from research and monitoring over the last 20 years since it was first identified in New Zealand. It describes the dynamics of major events and their consequences, and evaluates what is likely to happen in the future as aquaculture expands into new areas with known histories of this problem.

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Notable physiological and morphological effects of ball clay addition on bloom forming organisms *Pyrodinium bahamense*, *Gymnodinium catenatum* and *Alexandrium catanella*

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Mutual flocculation of algal cells and clay particles after clay addition is one of the promising remedial measures to counter the negative impacts of harmful algal blooms (HABs). However, very few studies explain the mechanisms, factors and effects of clay particles on algal physiology and morphology. This knowledge gap is the focus of this study. The effects of clay dosage, time, pH and ionic strength were investigated through batch adsorption experiments using ball clay (kaolinitic) and monocultures of *Pyrodinium bahamense, Gymnodinium catenatum* and *Alexandrium catanella.*. Results showed that the addition of aqueous clay slurry (0.25-1.0 g L^{-1}) removed 80-90% of the three species within 12 h. Additionally, cell lysis occurred within 30 min of exposure, as observed using Confocal Laser Scanning Microscopy (CLSM). Ball clay adheres to the cell surface thereby enhancing its stickiness which triggers cell-cell adhesion and the eventual disruption of the lipid bilayer of the algal cells and release of intracellular materials. Physiological and morphological effects of ball clay addition will be described for the three HAB species.

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Plankton Toolbox – open source software making it easier to work with plankton data

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Modern database systems make it possible to access large data sets on the occurrence of harmful algal bloom species. This gives insights into geographical distribution of species, bloom development etc. Data sets on plankton biodiversity and abundance including harmful algal bloom species may be time consuming to work with. We have developed Plankton Toolbox, an open source stand-alone application, to make the process easier. The software is mainly aimed at working with data from water samples analysed microscopically but the software is applicable also to other data. The workflow includes importing data, a quality control step, ways to filter out data for specific purposes, functions for aggregating data, plotting tools and some tools for statistical analyses. Importing and exporting of data in different formats is supported. For advanced statistical analyses exporting to other software is useful. The system is based on quality controlled species lists available at http://nordicmicroalgae.org but users are free to work with their own check lists. Lists on cell volume and carbon content of species come from the HELCOM-Phytoplankton Expert Group but users can also define their own lists. The software was developed using Python and runs on personal computers with Windows or MacOS. A Linux version is in development. The software is available at http://nordicmicroalgae.org/tools.

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Tracking changes in the Neuse River Estuary in North Carolina, USA from 1998-2013.

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In response to harmful algal blooms and fish kills on the Neuse River during the 1990s, a permanent 30% reduction in total nitrogen entering the estuary was implemented from late 1998 through 2003. Consistent taxonomic data on phytoplankton assemblages in addition to photic zone chemical (chlorophyll *a*, NO_x^-, total phosphorous, pH) and physical (dissolved oxygen, temperature) data collected by the North Carolina Division of Water Resources on the Neuse River from 1998-2013 were examined. Within this construct, this study focused on the role of nutrient reduction on population shifts of three harmful algal bloom taxa—the cyanobacteria *Cylindrospermopsis raciborskii* and the dinoflagellates *Karlodinium veneficum* and *Prorocentrum minimum* within the Neuse River.

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Seasonal observations from bio-optical HAB surveillance in the Ebro Delta, NW Mediterranean

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The high diversity among HAB species and the complexity of their distributional patterns necessitates regional observational approaches that cover a broad spectrum of temporal and spatial scales. In this study, we demonstrated that datasets from remote sensing by means of an *in situ* sensor system, complemented by analyses of taxa and toxins, can define HAB distributional patterns in the aquaculture area of the Ebro Delta, NW Mediterranean. Bio-optical techniques can serve to gain insights into distribution, adaptive strategies and habitat preferences of harmful taxa. Findings of this study show how an environmental observatory with a radiometric sensor system as key component contributes to effective surveillance of selected high biomass-forming taxa in the area. Yet it was also clearly shown that detailed knowledge on bloom characteristics is crucial for interpretation of bio-optical data. Comparative findings during the study period in spring/summer indicate that *Karlodinium* and *Dinophysis* species constitute alternative species types with different preferred habitats. Such understanding of bloom dynamics may be transferable to other embayments in a comparative approach, and eventually lead to the delineation of distinct habitat niches of harmful taxa. The integration of bio-optical and conventional discrete sampling for HAB surveillance allows for effective tracking of the presence and movement of HABs. From a scientific perspective, such systems approaches provide insights into environmental scenarios that favour HAB proliferation and would eventually lead to prediction of algal growth and distributional patterns. Future efforts should also consider participation of the general public in HAB surveillance and education programmes. Together these strategies offer the opportunity to optimise and direct public and private responses to the harmful event and to mitigate undesirable impacts.

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Water quality improvement by the polychaete rockworm *Marphysa sanguinea* (Montagu) in the integrated culture with olive flounder

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Aquaculture is growing rapidly all over the world and the increasing number of fish culture industries has begun to create serious environmental problems due to the impact caused by fish farming wastes. Based on the successful seed production of the polychaete rockworm *Marphysa sanguinea*, three trials have been conducted to test the water quality improvement and growth performance in the rockworm settlement tanks receiving wastes from olive flounder rearing tanks. In comparison between culture systems, the pure production of the worm was 1.5 times higher in flow-through system than in semi-recirculating system. Different effects on the removal efficiency were appeared both in culture systems and among treatments. In flow-through system, the highest removal efficiency showed in TN (56 %) in G2 (0.6~1.5 g/initial weight), TP (59%) in G1 (<0.5 g), TSS (86%) in G2, and COD (30%) in G3 (1.6~2.5 g). In semi-recirculating system, the highest removal efficiency was in TN (63%) in G2, TP (53%) in G3, TSS (80%) in G3, and COD (21%) in G2. These results suggest that the rockworm *M. sanguinea* is an excellent candidate for integrated aquaculture and nutrient recycling including the removal of organic wastes in land-based systems.

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Alexandrium and risk management within the Scottish phytoplankton monitoring programme

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The potentially harmful genus *Alexandrium* has been detected in Scottish coastal waters during routine regulatory monitoring of shellfish harvesting areas. *Alexandrium* can be highly toxic, particularly in spring and early summer, and dense blooms are not required before there is cause for concern. Thus, the current trigger level to instigate additional biotoxin testing is set at 20 cells per litre. Although a close link between *Alexandrium* and PSP toxins can be frequently demonstrated, it has often been the case that *Alexandrium* blooms do not correspond to elevated shellfish toxicity. Both toxic and non-toxic species/strains have been observed around Scotland and frequently co-occur in the water column. In an effort to reduce the burden on small businesses, recent guidance issued to harvesters has advised that classified shellfish growing areas be moved to a higher alert status if *Alexandrium* is present in seawater at a concentration of 40 cells per litre or greater, or has been in the preceding two weeks. We examine PSP toxic events over a five year period, where quantifiable levels of toxins were reported in common mussels, to determine whether a change from the current trigger level would have resulted in potentially harmful toxic events being missed. Data suggest that if the trigger level was increased to 40 cells per litre, it is unlikely that toxins would have exceeded regulatory limits before action was taken. The analysis highlights the importance of weekly phytoplankton monitoring over the high risk period.

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Assessment of microcystin in freshwater fish in Alberta, Canada

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Microcystin (MC) is the most frequently occurring cyanobacterial toxin in Alberta's fresh water lakes. In other parts of the world, MCs have been found in some fish, mussels and shellfish raising the possibility of food as a route of exposure to humans. The present study to determine MC concentrations in sportfish was conducted as part of a public health monitoring program. A total of 561 fish representing seven different species were harvested between August and October 2012 from 12 lakes exhibiting blue-green algae blooms to an extent warranting posting of recreational water use advisories. The sampling period coincides with the highest expected water concentrations of MC based on past observations. Muscle samples (357 fillet subsamples) were analysed for MC-LR, RR, YR, LW and LF using a sensitive liquid chromatography/tandem mass spectrometry (LC-MS/MS) method. None of the muscle samples had detectable levels of MC as quantified by LC-MS/MS. These findings suggest that MC does not accumulate appreciably in muscle of fish from Alberta's bloom-prone north-temperate lakes and that health risk associated with the consumption of fish fillet from these lakes is low. One explanation for the absence of MCs in fish muscle could be the seasonal presence of algal blooms during the short summer periods, resulting in limited exposure of freshwater fish under Alberta's climate conditions.

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Chlorophyll distribution and abundance in a small embayment with Harmful Algal Bloom (HAB) occurrence in Murcielagos Bay, Philippines

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A bloom of the dinoflagellate *Pyrodinium bahamense* var. *compressum* was recorded during the February 2011 survey of Murcielagos Bay, Philippines. This has been revisited three times after this occurrence all during the Northeast monsoon. The bay is relatively small and shallow with an average depth of 5 meters, maximum depth of 50 meters at its mouth, and a narrow and shallow passage towards a smaller embayment located at the south called *Sapang Dalaga*. The shallow bathymetry of the bay creates very weak current flows that are both tide and wind driven, most especially inside *Sapang Dalaga*. Interestingly, in *Sapang Dalaga*, high chlorophyll concentrations were repeatedly observed to be situated mostly near the bottom of the water column. To associate the abundance and distribution of phytoplankton in the water column, a three-layer hydrodynamic and water quality models using bathymetry, current flow and recorded field data of chlorophyll concentrations were developed. The models calculated the longest residence time at the third layer or bottom layer of the water column and coincided with actual field data where chlorophyll concentrations were also highest. During the 24-hour monitoring in November 2013, recorded field chlorophyll concentrations confirmed direct correlations with salinity, density, and total nitrate and nitrite concentrations. In summary, the hydrodynamic configuration, stratification, and nutrient availability played a significant role in the abundance and distribution of chlorophyll. This is essential in understanding the many factors that might influence the abundance and distribution of various harmful algal bloom species in Murcielagos Bay, Philippines.

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Ecotoxicological characterization of 3 different lagoons of French Polynesia : Tikehau, Kaukura and Mangareva.

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In Pacific Islands Countries and Territories (PICTs), seafood is a staple nutritive and sometimes the only economic resource. Seafood-borne diseases have been on the increase since last few decades, resulting from bio-accumulation in the food chain of marine biotoxins naturally produced by several species of phytoplankton during microalgal or even cyanobacterial blooms. In French Polynesia, Ciguatera Fish Poisoning (CFP) is the most prevalent seafood intoxication due to the consumption of contaminated coral reef fishes but also of marine invertebrates like giant clams. Furthermore, to cope with ciguatoxic risk in seafood products, populations can be forced to adapt their food habits by increasing their consumption of manufactured products as well as pelagic fish.
Ecotoxicological characterization of three different islands has been evaluated in different areas of the lagoons more or less impacted by natural or anthropogenic factors: Tikehau and Kaukura atolls (Tuamotu Archipelago) and Mangareva Island (Gambier Archipelago). Indeed, the mean annual incidence of CFP cases per 10,000 inhabitants from 2007 to 2013 was 8, 137 and 406 in Tikehau, Kaukura and Mangareva, respectively. Regarding the presence of dinoflagellates and cyanobacteria, biodiversity was great in Mangareva Island in contrast to Tikehau and Kaukura atolls. Survey of ciguatoxins in the food web has been done by screening phytoplankton, fish and marine invertebrates likely to contaminate humans. Likewise, the impact of CFP on eating habits and human health has been measured. Results between those 3 islands will be compared.

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