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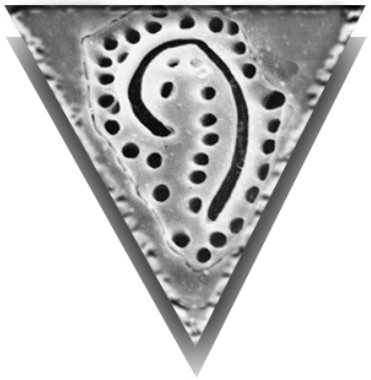

Ninth Conference
TASMANIA
2000

7-11 February 2000
Wrest Point Convention Centre, Hobart,
Tasmania, Australia

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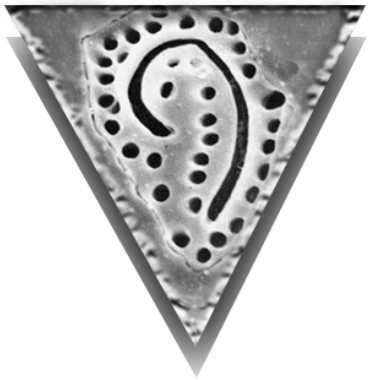

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Harmful ALGAL BLOOMS

Ninth Conference
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2000

ABSTRACTS OF

ORAL COMMUNICATIONS

PROMOTION OF CYST FORMATION IN THE TOXIC DINOFLAGELLATE *ALEXANDRIUM* (DINOPHYCEAE) BY NATURAL BACTERIAL ASSEMBLAGES

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The relationship between the abundance of the toxic marine dinoflagellate *Alexandrium* spp. and cyst formation promoting bacteria (Alex-CFPB) was investigated in the water column of Hiroshima Bay (Japan) during 1997 – 98. All the sea water fractions collected from 5m depth where the density of *Alexandrium* cells was highest, and which also contained the bulk of planktonic bacteria, promoted cyst formation of *A. catenella* (Whedon and Kofoid) Balech. This promotion was not caused by nutrient limitation. The number of Alex-CFPB in the sea water samples, that was calculated by means of the most probable number (MPN) method, had a clear positive correlation with the abundance of *Alexandrium* spp. during blooms. 31 bacterial strains that have *Alexandrium* cyst formation promoting activity ("Alex-CFPB") were isolated. Population structure and genetic diversity of "Alex-CFPB" were analyzed by means of restriction fragment length polymorphism (RFLP) and partial sequences of the 16S ribosomal RNA genes. Fourteen ribotypes, A – N types, were observed among 31 strains of "Alex-CFPB" by means of RFLP with several restriction enzymes. Bacterial strains of ribotype A were dominant in the "Alex-CFPB" assemblages during the peak and termination periods of the *Alexandrium* bloom. The 16S rDNA based phylogenetic tree of the nine ribotypes among them showed that these fell within the alpha subdivision of the class proteobacteria. When encystment promotion activities of ribotype -A and -C isolates were analyzed in detail considering their effects on the algal growth, the encystment efficiencies (cyst yields/ maximum cell density) under the inoculation of each bacterium were almost 13 and 16 times as much as those under bacteria free conditions, respectively. These results suggest that Alex-CFPB play a significant role in the process of encystment and bloom dynamics of *Alexandrium* in the field.

YESSOTOXIN: A POWERFUL NEW TOOL FOR THE STUDY OF SIGNAL TRANSDUCTION

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Yessotoxin is a generic name for a group of compounds recently discovered and chemically characterized. These compounds pose a classification problem regarding their action, since, although they are considered diarrheic toxins because of their lipophilic nature, their effect is non diarrhogenic. Yessotoxin has been reported to be cardiotoxic, but there is no information about its mechanism of action. We present here preliminary data about the mechanism of action of these toxins in fresh human lymphocytes, as an easily available probe to detect the action of these compounds in humans. The action is mediated by modulation of cAMP and cytosolic calcium levels. Yessotoxins increases cytosolic calcium levels by increasing the release from intracellular reservoirs and the influx of external calcium. The most striking result we have gathered is that yessotoxin inhibits cAMP levels in resting lymphocytes. This effect is very intense, calcium-dependent and seems to be mediated by inhibition of adenylate clyclase or G proteins, an study we are currently performing. This inhibitory effect is not related to the inhibition of protein kinase A or phosphodiesterases. Overall, yessotoxin seems to be a powerful toxin, with a very interesting mechanisms that might be used to study signal transduction.

THE ECOLOGY AND OCEANOGRAPHY OF TOXIC *ALEXANDRIUM* BLOOMS IN THE GULF OF MAINE: RESULTS FROM THE ECOHAB-GOM PROGRAM

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The ECOHAB-GOM program was initiated to address fundamental mechanisms underlying *Alexandrium* blooms in the Gulf of Maine: 1) the source of the *Alexandrium* cells that appear in the fresh water plumes in the western Maine coastal current (WMCC); 2) *Alexandrium* cell distribution and dynamics in the eastern Maine coastal current (EMCC); and 3) linkages among blooms in the WMCC and the EMCC. Utilizing a combination of numerical modeling (highlighted in this presentation), hydrographic, chemical, and biological measurements, moored and drifting current measurements, and satellite imagery, a team of 14 investigators from 9 institutions is attempting to characterize the structure, variability and autecology of the major *Alexandrium* habitats in the Gulf of Maine.

Intensive, small-scale field surveys in 1998 near the outflow of the Kennebec River, a putative "source region", documented an offshore source of cells, presumably from cysts germinating in deep basins. Model simulations of cyst germination (regulated by temperature, light, and an endogenous clock) were used to provide a time- and space-varying input of cells to the coupled physical/biological model of the coastal current system. "Hind-cast" model simulations are consistent with field observations during two bloom seasons. These simulations also led to the development of a conceptual model of how germinated cells in offshore waters may become entrained into the coastal current. Also in 1998, large-scale surveys between Casco Bay and the Canadian border suggest that *Alexandrium* dynamics in the EMCC are regulated by nutrient availability and mixing depth (light) in a manner that fosters offshore growth and accumulation. ECOHAB-GOM is thus a combined modeling/observational program following an approach commensurate with the multiple scales and oceanographic complexity of paralytic shellfish poisoning phenomena in the Gulf of Maine. Updates of model and cruise results can be found at: http://crusty.er.usgs.gov/ecohab/.

DEPURATION OF PSP TOXINS FROM GREEN MUSSELS, *PERNA VIRIDIS*

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The depuration of PSP toxins from naturally- contaminated green mussels, *Perna viridis* harvested from commercial shellstock culture sites in Limay, Bataan, Philippines from February 1998 – May 1999 was studied. The effects of varying salinities from 20-35 ppt at 20-25 C of the recirculating seawater of the ultraviolet (UV) depuration set-up during the 72h continuous shellfish purification to eliminate PSP toxins and microbial contaminants from the mussels were monitored. A seawater salinity-temperature combination of 31-35 ppt at 20-25 C was observed to be most effective condition in eliminating PSP toxins and microbial contaminants from the mussel meats during depuration. Percent decrease of PSP toxin burdens in whole mussel meats at salinity ranges of 20-25, 26-30, and 31-35ppt, after 72h purification as determined using standard mousebioassay, were established to be approximately 13, 8, and 38 %, respectively. The relative anatomical distribution of PSP toxins in depurated green mussels showed the following trend: adductor muscles > digestive tissues and gonads > gills and mantle > foot. Counts of indicator microorganisms of fecal origin including coliforms and *Escherichia coli* from naturally-contaminated green mussels showed > 80% decrease after the application of the test purification procedure.

ARE *PYRODINIUM* BLOOMS IN THE SOUTHEAST ASIAN REGION RECURRING AND SPREADING? A VIEW AT THE END OF THE MILLENIUM

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Pyrodinium bahamense (var. *compressum*) has been the only dinoflagellate species that has caused major public health and economic problems in the Southeast Asian region for more than 2 decades now. Blooms of the organism have been reported in Malaysia, Brunei Darussalam, the Philippines and Indonesia. The ASEAN-Canada Red Tide Network has recorded 31 blooms of the organism in 26 areas since 1976 when it first occurred in Sabah, Malaysia. As of 1999, the most hardly hit country has been the Philippines which has the greatest number of areas affected (16) and highest number of PSP cases (about 1,990). Cysts of the *P. bahamense* var. *compressum* have been reported in three bays in the Philippines and in one bay in Indonesia; but it is very likely, however, that they could be present in adjacent bodies of water/countries where sampling and analysis of sediments have not been done. Research on sediment-dated cores should be undertaken in addition to those that relate to the role of cyst beds in the organism's bloom dynamics.Most of the data and information useful to understand *Pyrodinium* bloom dynamics have come from harmful/toxic algal monitoring and research that have developed to different degrees in the various countries in the region affected by the organism's bloom. Molecular genetic studies to distinguish population between and within the Atlantic and Indo-West Pacific areas need to be undertaken. Such studies will be helpful in elucidating questions relating to the distinction between *P. bahamense* var. *compressum* and *P. bahamense* var.*bahamense*; and the origin and spread of *P. bahamense* blooms, particularly in the Indo-West Pacific region.Regional collaborative research and monitoring efforts can help harmonize local data sets and ensure their quality and availability for comparative analysis and modeling. Temporal patterns of the blooms at local and regional scales, possible signals and trends in the occurrence/recurrence and spread of *Pyrodinium* blooms and possible role of climactic changes as El Niño event can then be detected; and predictive models developed.

ON A BLOOM OF *CHATTONELLA* IN THE NORTH SEA/SKAGERRAK IN APRIL-MAY 1998.

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In April-May a mass occurrence of *Chattonella* aff. *verruculosa* (Rhaphidophyceae) caused brownish sea in parts of the Skagerrak and the North Sea. Both wild fish and fish in pens were killed during the bloom. In April, *Chattonella* grew to a dense population in an area to the north of Skagen in the Skagerrak. On April 12 it was also common outside the Limfjord at the west coast of Denmark. In the very beginning of May (1-3) significant mortality among large Atlantic salmon in pens were reported by fish farmers at the south-west coast of Norway. Later in May (7-19) very high concentrations of *Chattonella* were recorded all along the west coast of Denmark, from the Jammerbukt and at least to the Esbjerg-area. This could partly be a spreading from the Skagen-area via an unusual, temporary south-west current, but in situ growth of algae seen along the west coast of Denmark in April could have contributed as well. The bloom developed in water bodies rich in nitrate, most probably brought to the Skagen-area from the southern North Sea. High N:P atomic ratios in particulate material, mainly consisting of Chattonella, collected by the end of the bloom, pointed to phosphor-limitation of the bloom. The number of common zooplankton collected by net (180 μm) in the blooming area was small. This may have been due to zooplankton avoiding the *Chattonella* bloom, which again may have led to a low grazing-pressure. This was, to our knowledge, the first record of this species in European waters, and world-wide, the first report on fish mortality associated with this species.

FEEDING RESPONSE OF KRILL TO THE TOXIN PRODUCING DIATOM *PSEUDO-NITZSCHIA*

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The interaction between toxic diatoms and their zooplankton grazers, as well as the food web transport of these toxins through food chain, are poorly known. Increasingly, attention is being focused on the trophic fate of the toxin produced by various species of *Pseudo-nitzschia*, which often bloom in Monterey Bay, California. In the food web of Monterey Bay, euphausiid crustaceans (krill) are important grazers of diatoms and are major prey for various organisms. To determine how heavily krill are feeding on local *Pseudo-nitzschia* species, we initially compared krill gut contents to diatom populations in samples collected from Monterey Bay, CA in July 1998. Examination of samples by scanning electron microscopy indicated that krill heavily consume *Pseudo-nitzschia*. Secondly, we conducted experiments to determine krill grazing rates on toxic diatoms in laboratory conditions. Krill were collected from Monterey Bay in May 1999 and fed clones of locally isolated *Pseudo-nitzschia multiseries*. Interestingly, each krill specimen consumed about cells $5x10^4$ in 6 hours. We still need to better understand the extent of krill exposure to toxic species and whether krill can transfer the toxin to higher trophic levels. Based on grazing experiments and field data, we estimate that each krill is capable of transferring 75 ng domoic acid (DA)/ krill/ hour to higher trophic levels. These results provide a perspective that will ultimately help us to understand toxin transfer from toxic blooms, which appear to be a rising threat to the health of coastal environments.

EFFECTS OF IRON ON DOMOIC ACID PRODUCTION BY *PSEUDO-NITZSCHIA MULTISERIES*

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Domoic acid (DA), the responsible agent for Amnesic Shellfish Poisoning, contains several carboxylic acid residues that could potentially bind trace metals such as iron. To investigate if DA production was affected by the iron status of the cell, replicate cultures of the diatom *Pseudo-nitzschia multiseries* were grown under trace-metal clean conditions containing 0, 0.12 μM and 11.7 μM added iron. Cell growth was monitored by in vivo fluorescence, extracted chlorophyll, and visual cell counts; DA production was measured by the FMOC HPLC method. All three iron treatments showed similar initial growth rates and resulted in stationary phase densities of greater than 10^5 cells/mL after 10 days in culture. In contrast, DCMU-enhanced fluorescence indicated that the cultures without added iron were iron-limited by day 18, as evidenced by an Fv/Fm ratio of 0.2 as compared to the iron-replete cultures (11.7 μM Fe) with an average Fv/Fm ratio of 0.5 throughout the culture period. Equally dramatic changes were seen in the total chlorophyll per cell and the mean DA production per cell. Cultures without added iron showed a marked drop in the cellular chlorophyll a content in stationary phase and never produced more than 5 pg DA/cell (<1000 ng/mL). In contrast, iron-replete cells contained 5-10 times more chlorophyll per cell, and DA production increased steadily throughout stationary phase to nearly 50 pg/cell (5500 ng/mL). The intermediate level of added iron (0.12 μM) showed intermediate results: the Fv/Fm ratio and chlorophyll content varied considerably throughout stationary phase and DA reached 20-25 pg/cell (~3500 ng/mL). These results indicate that the lack of available iron strongly inhibits the ability of P. multiseries to produce DA. This decrease in production suggests that DA is not produced as a chelator to increase the availability of iron to the cells. The cause of this decrease is unknown, but the biosynthesis of DA requires both nitrogen and energy, two resources that are likely to be limiting under iron-deficient conditions.

THE EFFECT OF LIPOPOLYSACCHARIDE AND MICROCYSTIN-LR ON GLUTATHIONE S-TRANSFERASE ACTIVITIES IN FISH

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Lipopolysaccharides (LPS) are produced by Gram negative bacteria including cyanobacteria. Bacterial LPS and their biological activities are well studied in relation to mammals, with particular reference to human medicine. Cyanobacterial LPS are less well studied and though they may be of lower toxicity to rodents than the LPS of *Salmonella* , they can be found in large quantities in cyanobacterial blooms. Laboratory purification by hot phenol extraction suggests that LPS may account for between 0.1-1% of cell dry weight of an axenic culture, and that toxicity according to *Limulus* assay varies between strains. Natural blooms are not found under axenic conditions, often they have bacteria associated with them, including coliforms. Commercially available *Salmonella* and *E.coli* LPS and three cyanobacterial LPS, (1 laboratory culture, 2 natural blooms) were used in the investigations. All LPS preparations strongly inhibited microsomal glutathione S-transferase (GST; EC 2.5.1.18) in zebra fish p6 stage embryos in vivo: enzyme activity was reduced from 0.502 to between 0.064 and 0.315 nkat.mg-1 protein. Soluble GST showed no inhibition in response to *Salmonella* or *E.coli* LPS, however activity was significantly reduced from 1.045 to between 0.186 and 0.216 nkat.mg-1 protein after exposure to all cyanobacterial LPS. When exposed to both MC-LR and LPS the microsomal GST activity in all exposures was reduced, soluble GST activity was reduced only in the cyanobacterial LPS-treated embryos. The results will be discussed in the context of microcystin detoxication and possible implications for fish and animal health.

THE HUON ESTUARY, SOUTH EAST TASMANIA: AN INTEGRATED APPROACH TO *GYMNODINIUM CATENATUM* BLOOM DYNAMICS

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The Huon Estuary is a major estuary in south-east Tasmania draining a catchment of temperate rainforest and rural/small urban areas. It is also the main waterway for aquaculture in the region, supporting an industry producing Atlantic salmon, mussels, oysters and abalone, with annual production approaching $100 million. Blooms of *Gymnodinium catenatum* were first recorded in 1986. Since then there have been irregular blooms, sometimes very extensive throughout the estuarine and coastal waters of south-east Tasmania, with resultant closures of shellfish leases and management problems for the Atlantic salmon industry. As part of a large multi-disciplinary study of the Huon Estuary we investigated the phytoplankton dynamics including *G. catenatum* blooms between 1996 and 1999. The study incorporated conventional estuary-wide surveys as well detailed weekly monitoring at 5 stations, including use of *in-situ* automated continuous profiling instruments with measurements of key physical and chemical parameters, including temperature, salinity, fluorescence, pigments, and dissolved oxygen. Against a background of small flagellates there were spring and summer diatom blooms and early summer to autumn dinoflagellates blooms. During summer 1996 dinoflagellates were absent; in contrast in summer and again in autumn of 1997/98 and 1998/99 there were extensive blooms of *G. catenatum* .Between these blooms *Ceratium* spp. and diatoms bloomed. Intriguing differences between the summer and autumn blooms of *G. catenatum* were the consistent presence of sexual stages, including resting cysts during the first bloom, but not in the second, suggesting a more significant role for resting cysts in *G. catenatum* blooms than previously thought. The blooms did not appear to be macronutrient driven, but the physics of the estuary was important. While blooms could develop and be maintained under only weakly stratified water column conditions a stable water column enhanced intense bloom development, and bloom decline was promoted by a large oceanographic event in one case and declining temperatures below 12 degrees Celsius in another. A key to success of *G.catenatum* within the flushing regimes of the estuary appears to its strong diurnal vertical migration. This study has demonstrated some of the key factors regulating *G.catenatum* blooms in the Huon Estuary, as well as showing the importance of fine scale continuous monitoring to resolve the details of bloom dynamics.

EVOLUTION AND BIOGEOGRAPHY OF THE *GYMNODINIUM CATENATUM* SPECIES COMPLEX: A MULTIDISCIPLINARY APPROACH

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The known global distribution of the distinctive PSP-producing dinoflagellate *Gymnodinium catenatum* has increased dramatically in the last few decades and it is now reported from every continent. Whether this represents increased recognition of a widespread cryptic species or recent dispersal from a source population is not clear. Recent studies have shown that the distinctive microreticulate cysts characteristic of *G. catenatum* are also produced by two distinct but related non-toxic species, *G. nolleri* Ellegaard et Moestrup and *G. microreticulatum* Bolch et Hallegraeff. Consequently, previous cyst reports may have been mistakenly attributed to *G. catenatum* or include more than one species. Resolving these distinct species has not only clarified the current distribution of *G. catenatum* but also provides an opportunity to examine the evolution and biogeography of the complex.

Interbreeding, biochemical, and molecular genetic comparison of *G. catenatum* strains from Japan, Spain and Portugal and Australia studies indicate a high level of variation within populations, consistent with a sexually outbreeding species. Small-scale geographic and temporal clustering of Tasmanian strains by isolation location and bloom year indicates that genetic exchange between neighbouring estuaries is limited and that Tasmanian *G. catenatum* blooms are composed estuary bound sub-populations. Japanese and Spanish/Portuguese strains can be resolved into distinct clusters which are, surprisingly, more closely related to each other than either population are to the Australian cluster. The probable source for Australian *G. catenatum* remains unclear, however, genetic and biochemical data support the secondary relocation of Tasmanian populations to mainland Australia, possibly via a domestic shipping vector.

Studies of LSU-rDNA sequences show that the *G. catenatum* complex is a distinct monophyletic lineage arising from the base of the loop-apical grooved group of gymndinioids. Within the complex, *G. microreticulatum* diverges earliest and shows abundant LSU-rDNA sequence variation between populations; *G. nolleri* and *G. catenatum* diverged much more recently, with *G. catenatum* exhibiting no sequence variation among strains from Australia, Hong Kong, Japan, Spain and Uruguay. Comparing nuclear volumes indicates that the complex may have evolved from a *G. microreticulatum*-like ancestor by a series of polyploidy events. A broad re-examination of the fossil dinoflagellate cyst record in light of recent molecular phylogenetic data suggests a common ancestor of the *G. catenatum* complex evolving around the end of the Cretaceous, with the *G. catenatum* lineage diverging around 130-140 Mya and that the *G.nolleri* and *G. catenatum* lineages diverging about 16-19 Mya. The known modern distributions of the complex suggest a European origin for *G. catenatum*, followed by dispersal to other continents within the last 25 thousand years, either by natural processes, human means, or a combination of both.

DIFFERENTIAL SENSITIVITY AND UPTAKE OF PSP TOXINS WITHIN AND BETWEEN SOFTSHELL CLAM (*MYA ARENARIA*) POPULATIONS FROM ATLANTIC CANADA

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This study compared the individual responses to paralytic shellfish poisoning (PSP) toxins of *Mya arenaria* from two populations with contrasting histories of toxin exposure: Lepreau Basin, Bay of Fundy, New Brunswick (NB) (annual, recurrent PSP outbreaks) and Lawrencetown estuary, Nova Scotia (NS) (no previous toxin exposure). Clams were exposed to a high-toxicity strain of *Alexandrium tamarense* (PR18b; 60 pg STXeq/cell), under identical laboratory conditions for 2-3 weeks. Remarkable differences were observed in behavioral (burrowing capacity), and physiological responses (feeding rate on *Alexandrium* cells, tissue toxin uptake, and in vitro block of the action potential in isolated nerves exposed to saxitoxin). Percent burial (4% vs. 86% in NS and NB populations respectively after 24 h of exposure) and average clearance rates were significantly lower (4 to 8x) in NS than NB clams. Toxicities were significantly lower (up to 10-fold in viscera) in sensitive NS clams than in resistant NB clams. The former experienced cumulative mortality rates of up to 24%, which started after one week of toxin exposure, whereas NB clams exhibited negligible or no mortalities. Marked differences (more than an order of magnitude) were also observed between dominant phenotypes of the test populations (sensitive NS vs. resistant NB individuals, based on burrowing response at 24 h). These were observed during toxification (4-6 days of exposure), and after 16-20 day-depuration on non-toxic algae (in this case survivors were selected for testing). All NS clams exhibited partial nerve block at 10^{-6} g STX/ml, and most were fully blocked within 20 sec at 10^{-5}. In contrast, most NB clams displayed no effect even at 10^{-5} and required 4 to 7 min. of exposure to induce full nerve block at 10^{-4} g STX/ml; some showed only partial block at 10^{-4} g STX/ml. Such intraspecific differences in sensitivity and toxin accumulation have important ecological and biotoxin monitoring implications, and suggest that genetic adaptation to toxins via natural selection of more resistant individuals may occur in areas recurrently affected by PSP.

THE TOXIC *PFIESTERIA* COMPLEX

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Recent outbreaks of *Pfiesteria piscicida* and a second known toxic *Pfiesteria* species have provided a compelling illustration of strong linkages between fish kills and subtle but serious impacts on human health. These two species thus far comprise the toxic *Pfiesteria* complex (TPC). Toxic strains of TPC species show strong attraction to live fish; toxicity triggered by the presence of fresh materials from live fish; and production of bioactive compounds that cause fish stress, disease and death. They also have a complex life cycle with multiple amoeboid as well as flagellated stages. TPC species are eurythermal and euryhaline, with optima for toxic activity at > 22oC and 15 psu. Their prey span the estuarine food web from bacteria to mammalian tissues. Toxic Pfiesteria outbreaks are known thus far from poorly flushed, nutrient-enriched areas of the Albemarle-Pamlico (epicenter, 88 events, > 1 billion fish affected) and Chesapeake (3 events, 30,000 fish affected) of the mid-Atlantic U.S. TPC species can be strongly stimulated by both N and P enrichments, both directly (organic N, P uptake; and inorganic nutrient uptake by kleptochloroplastidic strains) and indirectly (mediated through algal prey). Water-soluble putative toxin produced by fish-killing *Pfiesteria* cultures has been partially purified based on c-fos-luciferase reporter gene assay-guided fractionation. This component kills fish and causes a concentration-dependent, prolonged elevation of cytosolic free calcium in GH4C1 pituitary cells and in primary cultures of neurons isolated from rat hippocampus. A hydrophobic toxin component has promoted edema and sloughing of fish epidermal tissue. Human health impacts from contact with water or inhalation of air over areas with actively toxic TPC populations have included respiratory, visual, and neurological impairment, especially severe neurocognitive impairment manifested as mostly reversible short-term memory dysfunction. Moreover, accumulating evidence indicates that these dinoflagellates can cause serious chronic impacts on human as well as fish health.

ENVIRONMENTAL AND GENETIC FACTORS REGULATING PRODUCTION OF POLYETHER TOXINS IN MARINE DINOFLAGELLATES

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Polyether toxins are produced by many species of marine dinoflagellates, comprising approximately a dozen genera. These polyether compounds are responsible for certain human intoxication syndromes linked to seafood consumption (ciguatera, DSP, NSP), and also include "fast-acting toxins" of poorly defined human health significance (e.g., gymnodimine, spirolides). Despite recent advances in structural elucidation, relatively little is known about the structural/functional relationships of these secondary metabolites derived via polyketide biosynthesis. Comparison of toxin profiles among natural dinoflagellate populations typically reveals a high degree of structural polymorphism and geographically distinct patterns. Yet the toxin spectrum is usually preserved upon transfer into clonal culture, and tends to be quite refractory to environmental perturbations – this suggests a strongly defined genetic template. However, the toxin cell quota may vary markedly over the culture cycle in response to physiological changes. The metabolic cascade leading to the synthesis of DSP toxin derivatives by the benthic dinoflagellate, *Prorocentrum lima*, and spirolide production by the planktonic species, *Alexandrium ostenfeldii*, has been investigated using photoperiod-induced cell synchronisation techniques. The polyether toxins are constitutively produced – they are not classic "stress" metabolites. Current efforts are focused on establishing the timing and sequence of key cell cycle events involved in the biosynthesis of polyether toxins. Although it is not possible to definitively ascribe a functional role to the polyether toxins, and gene regulation of toxin production remains poorly understood, hypotheses concerning their evolutionary significance and biogeographical distribution must be addressed.

OCCURRENCE AND DISTRIBUTION OF A NEWLY DESCRIBED *GYMNODINIUM BREVISULCATUM* SP. NOV. DURING THE 1998 SUMMER TOXIC OUTBREAKS ON THE CENTRAL EAST COAST OF NEW ZEALAND

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In the early 1998 summer, sporadic mortalities of fish and marine fauna occurred around the Wairarapa coast, central east coast of New Zealand. Over the period from January to early February 1998, more than 200 people were reported to have suffered from respiratory illness apparently originating from the build-up of the newly described *Gymnodinium brevisulcatum* sp. nov. in a nearshore bloom. By mid-February 1998, further human respiratory syndromes were reported in Hawke Bay to the north of the Wairarapa coast. Between mid-February and March 1998, an almost monospecific bloom (max. cell concentration recorded >33 million cells per litre) of the same new species occurred in Wellington Harbour, which is located to the southwest of the Wairarapa coast. This unprecedented bloom in the harbour killed a large number of fish, marine fauna, and seaweeds. Similar human respiratory distress, previously experienced along the Wairarapa coast, was also reported around Wellington. Over the period from January to March 1998 large scale changes in oceanographic conditions had been observed on the east coast of New Zealand. Results from a research voyage off the North Island east coast have shown that warmer subtropical water was found further to the south than usual, associated with a strong southward current. This is consistent with observations of warmer than usual satellite sea surface temperature during the period mid-January to mid-February 1998. Examination of seven across-shelf transects stretching around the entire east coast of North Island showed the presence of *G. brevisulcatum* sp. nov. in the warm, southwards-directed, offshore, East Auckland Current and East Cape Current. In February 1998, highest cell concentrations of *G. brevisulcatum*, however, were found in the southern section of relatively cool, less saline, northwards-directed, nearshore, Wairarapa counter current. The widespread occurrence of this toxic *G. brevisulcatum* during the 1998 summer seems to be related to a combination of downstream effect of weather changes associated with the El Nino pattern, and the dynamic interplay of two coastal current systems.

RAPID IDENTIFICATION OF MARINE ALGAE (RAPHIDOPHYCEAE) USING THREE-PRIMER PCR AMPLIFICATION OF NUCLEAR INTERNAL TRANSCRIBED SPACER (ITS) REGIONS

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The internal transcribed spacer (ITS) region of the nuclear rDNA was used to discriminate among cultured marine raphidophytic algal species of *Chattonella antiqua, C. subsalsa, Fibrocapsa japonica, Heterosigma akashiwo* and *Olisthodiscus luteus*. These wall-less algae can be very difficult to identify from natural water samples due to their fragile nature and pleomorphic morphology. Several of these algae have been associated with massive finfish kills throughout the world, therefore positive identification is of great importance. Species-specific diagnostic PCR fragment sizes resulted from three- primer amplification reactions and can reproducibly detect as few as five cells per sample. Recently, an active area of ecological research has been retrospective studies from archival material to determine population dynamics and species diversity. The use of these PCR primers with fresh cultures as well as archived material containing either formalin or Lugol s iodine has been successful. This is a valuable technique for detection and discrimination among marine Raphidophyceae particularly when cells can not be identified morphologically.

DINOFLAGELLATE RESTING CYSTS AS SEED BEDS FOR HARMFUL ALGAL BLOOMS

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We consider the possible extent to which dinoflagellate cyst-bed dynamics contribute to the seemingly unpredictable pattern of HAB occurrence. The following are defined: Population of motile cells in the bloom (MP1), initial population of cysts produced by MP1 (ICP), the viable cyst population from ICP (VCP) producing a new motile population (MP2), and the size and shape of the sedimentary system within which viable cysts may be transported (SS). Three basic "bloom strategies" are suggested: 1) HAB species without resting cysts (e.g. *Gymnodinium breve* in Florida) – MP2 is residual from MP1 surviving in the water column, independent of the sedimentary regime; 2) HAB species heavily dependent on seed beds (e.g. *Alexandrium tamarense* in higher latitudes) – these are most affected by the sedimentary regime where environmental factors can be identified at all stages with potential for producing greatly different values for MP2 from the same sized MP1 and SS; well suited to restricted parts of coastal environments (e.g. fjords, bays) with the closest possible isolation of discrete combinations of water bodies and sedimentary regimes, and with marked seasonality where cysts with long mandatory resting periods act as important over-wintering stages; and 3) (recognized from our observations of changes since 1995 in cyst assemblages in a newly constructed harbour on the otherwise exposed southwestern coast of Portugal) HAB species that produce large amounts of cysts but seemingly independent of seed beds (e.g. *Gymnodinium catenatum* along the southwestern coast of Europe/northwestern coast of Africa) – cysts probably excyst within a few days of formation, leaving only large amounts of empty cysts and non-viable cysts in sediments; probably function as category 1 above despite cyst formation. For categories 1 and 3, long term monitoring (> 30 years) offers the possibility to identify determining factors in hydrological and meteorological conditions (Stumf et al., 1997) that should help with HAB predictions. For category 2, cyst-bed dynamics need to be considered as an important scource of variance not usually accounted for (Eilertson & Wyatt, 1997), but this study suggests the need for better understanding of sedimentary processes than has been applied so far.

NOCTILUCA SCINTILLANS — AN INDICATOR OF COASTAL EUTROPHICATION?

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*Noctiluca scintillans ,*a large heterotrophic dinoflagellate, has been present in New South Wales coastal waters since 1860. *Noctiluca* was a minor component of the phytoplankton up until the last decade, but since then, *Noctiluca* blooms appear to have increased in frequency and intensity, with the majority of blooms recorded in recent years. There has been public concern that this increase in *Noctiluca* blooms may be a result of chronic sewage discharge from three deepwater ocean outfalls in the coastal waters surrounding Sydney. Such concern is consistent with the debate that *Noctiluca* maybe a coastal eutrophication indicator species. Over a one year period (1997-1998) at a long-term monitoring station off Sydney, *Noctiluca* was present year round with peak abundances coinciding with episodic slope water intrusions (and subsequent upwelling) during spring and summer. Extensive red tides of *Noctiluca* often succeeded diatom blooms (specifically *Thalassiosira* spp.) which were initiated by these intrusions. 80-90% of *Noctiluca* cells contained food particles and there was a positive linear relationship between abundance and the proportion of cells containing food. Prevalent food items within the vacuoles of *Noctiluca* were diatoms. Water samples collected north of Sydney also showed that highest numbers of Noctiluca occurred in areas predisposed to upwelling. Upwelling is likely to be the mechanism that promotes population growth of *Noctiluca* along the coast of N.S.W. Despite these findings, it is still difficult to disregard the direct or indirect effects of anthropogenic nutrients on the growth of *Noctiluca.* A long term assessment of physico-chemical dynamics in the water column off Sydney showed that there was no real change in phytoplankton biomass nor in uplifting/upwelling frequency in the last decade, yet the recent year round presence of *Noctiluca* is unprecedented for this region. A shift in the dominant diatom genera to *Thalassiosira* (believed to be the optimal food source of *Noctiluca*) concomitant with a rise in temperature and reduction in nitrate and phosphate levels may be the cause for the increase in *Noctiluca* numbers during this sampling year. The presence of a strong ENSO signal in 1997-1998 was shown in companion studies to dominate physico-chemical conditions, and thereby mask any anthropogenic effects. Future investigations need to incorporate this influence to resolve and partition climatic and anthropogenic signals.

HONG KONG'S WORST FISH KILL FROM A RED TIDE: (MARCH-APRIL 1998)

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The local press reported that the worst red tide-induced fish kill in Hong Kong's history had destroyed over 1,500 metric tons of maricultured fish stocks. The Hong Kong Government estimated the fish farmers' losses at HK$80 million (US$10.3 million), but fish farmers claimed the figure was at least HK$250 million (US$32 million). About 1,000 of Hong Kong's 1,500 fish farms were devastated by the *Gymnodinium mikimotoi* red tide. The red tide "spread out like an infectious disease," stated Hong Kong Fish Culture Association Chairman, Wong Yung-kan. Phytoplankton samples taken near the fish mariculture cages at Mo Tat Wan on 15 April 1998 contained over one million cells L^{-1} of *Gymnodinium mikimotoi.* At high densities, the *G. mikimotoi* produced a sticky mucus. When the mucus touched plant fibers or chitin or other dinoflagellates it stuck to them. When it made contact with the gill filaments of fish or shellfish it asphyxiated up to 90% of the impacted fish within 30 minutes. This rapid mortality gave rise to speculation that the slime contained a phycotoxin. The gills of the dead fish that were examined were coated with dinoflagellates trapped in their own slime. The reason that dinoflagellates produce copious amounts of a sticky mucus can be inferred from photographs taken at the peak of the 15 April bloom near Lamma Island where the Hong Kong mariculture industry was particularly hard hit. The photographs indicate that the secreted mucus sticks to inanimate objects such as plant fibers as well as to living objects such as the chitinous exoskeleton of copepods. The grazing efficiency of copepods with *G. mikimotoi* slime attached to their carapace is considerably reduced. Thus, mucus production may increase *G. mikimotoi* (dinoflagellate) survival by reducing the filtering efficiency of predatory copepods or by assisting dinoflagellates to form long chains or large mucus covered balls which are too large for most predators to handle.

MICROCYSTIN BIOSYNTHESIS: GENES, ENZYMES, REGULATION, FUNCTION

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Microcystins are cyclic hepatotoxic peptides consisting of 7 amino acids including Adda and other unusual amino acids. They are synthesized non-ribosomally by peptide synthetases. We identified DNA sequences with homology to conserved regions of peptide synthetase and polyketide synthase genes in the microcystin-producing strain PCC 7806 of *Microcystis aeruginosa.* The determined sequence of ca. 50kb comprises two gene clusters (operons), one harbouring peptide synthetase genes (mcyA,B,C), the other peptide synthetase genes and polyketide synthase genes (mcy D,E,F,G). The activities of polyketide synthases are expected to be required for the biosynthesis of Adda. The transcriptional activity of the genes has been studied under different environmental conditions. We inactivated two of the peptide synthetase genes and a polyketide synthase gene in strain PCC 7806 by genetic transformation and insertional mutation. The mutant cells obtained were unable to produce any variant of microcystin whilst maintaining their ability to synthesise other small peptides. Thus, the disrupted genes are specifically involved in the biosynthesis of microcystins. The mutant cells lack several proteins of high molecular weight including peptide synthetases which are hypothesized to form the microcystin synthetase complex. Genes encoding microcystin synthetases were found in all hepatotoxic strains and are absent in most non-toxic strains of *M. aeruginosa*. Possible roles for microcystin, as feeding deterrent against zooplankton or as having a function in intracellular metabolism, have been suggested as a result of mutant versus wild type analysis.

UPTAKE OF HUMIC SUBSTANCES BY THE TOXIC DINOFLAGELLATE *ALEXANDRIUM CATENELLA*

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Alexandrium catenella is a PSP producing dinoflagellate which blooms in organic-enriched (from terrigenous and anthropogenic sources) southern Australian waters. As partof a long term project examining mixotrophy and toxin producing phytoplankton, we conducted a series of experiments to test whether *A. catenella* utilises humic substances (HS) and how this affects cellular toxin content. Humic utilisation was tested in three different ways: by (1) monitoring growth, N, C, chl a, and toxin content of N-deplete *A. catenella* (axenic strain CCMP1598) cells in the presence of NO3, NH4, urea and HS; (2) measuring uptake of radioactively labeled humic substances (^{3}HS; isolated from seawater using XAD and subsequently labeled by Amersham); and (3) determining radioactive accumulation in *A. catenella* using microautoradiography . Cells in NH4 (15 μM) cultures died, but growth in NO3 and HS cultures was similar (0.5 d-1),with urea cultures having the lowest growth (0.2d-1). Toxin concentrations were low (maximum PSP toxins = 0.2 pM), with greater proportions of N-rich C-toxins compared to STX, NEO, and GTX toxins, but there was no apparent difference in toxin content between cells exposed to different N sources. In addition, C (300 – 430 pg) and N (50 – 90 pg) quotas of cells after 14 days were similar in the different N treatments, indicating HS utilisation by *A. catenella*.. Radioactivity in *A.catenella* cells increased from 1 – 7 disintegrations per cell [dpc] during the ^{3}HS incubation, with greatest accumulation during the first 24 h (including 1 light and 1 dark period). NO3 adapted cells also accumulated ^{3}HS, with a delay compared to HS adapted cells. Microautoradiography showed that most of the radioactivity was located inside the cell (active uptake), with the remainder being associated with the cell surface (passive adsorption). From these results we conclude that dinoflagellates take up HS directly, that these compounds are a potential source of C and / or N, and that dissolved organic N uptake involves a switch mechanism or simultaneous uptake with inorganic N.

A SINGLE-CELL IMMUNOASSAY FOR PHOSPHATE STRESS IN *PROROCENTRUM MINIMUM*

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Which nutrient or factor limits primary production in different marine waters continues to be the subject of research and debate among oceanographers. Current techniques for studying phytoplankton physiology such as measurements of biochemical activities, nutrient addition bioassays and determination of photosynthetic efficiency are generally capable of assessing the physiology of the bulk community but suffer from a lack of specificity. Thus, interest is increasing in the development of single-cell methods for monitoring in situ physiology. Cell-specific techniques are particularly helpful for studying and monitoring populations of harmful species in mixed assemblages.An antibody-based assay for identifying phosphate stress in the dinoflagellate *Prorocentrum minimum* was developed. Antiserum was generated against a cell-surface alkaline phosphatase purified from *P. minimum.*Western screening indicated that the antiserum reacted with phosphate-stressed cells but not nitrate-stressed or phosphate-replete cells in culture. Immunodepletion using either pre-immune or primary antisera cross-linked to agarose beads confirmed the target protein was an alkaline phosphatase. At this juncture the antiserum appears specific for phosphate-regulated proteins in *P. minimum* as there is no discernible cross reaction with closely related *P. micans* in western blots. In addition to westerns an immunofluorescence protocol was developed to screen field populations. This technique complements our recent development of ELF-97-labeling as a tool for examining *P. minimum* phosphate stress in field populations.This is the first antibody-based method developed for monitoring cell-specific physiology in a dinoflagellate. The method described here may serve as a model for developing similar tools in other species of dinoflagellates.

GENETIC ANALYSES OF *DINOPHYSIS* SPECIES ISOLATED FROM NORWEGIAN WATERS

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Dinophysis species may contain Diarrhetic Shellfish Toxins (DST). They occur all along the Norwegian coast and may in some regions prevent harvesting of mussels several months each year. The content of toxins seems to vary considerably between and within species. Also the morphology is very variable within some species and the species-delineation can at times be unclear. We have examined the phylogenetic relationship of four *Dinophysis/ Phalacroma* species (*D. acuminata,D. acuta, D. norvegica* and *P. rotundatum*) isolated from Norwegian waters inferred from the 18S ribosomal RNAgene. The genetic variability within the species *D.acuminata* and *D. norvegica* was examined by analysing the first internal transcribed spacer (ITS1) in 5 isolates per species collected at different times of the year and from two localities off the coast of southern Norway. The three photosynthetic species *D. acuta, D. acuminata,* and *D.norvegica* were very similar within the 18S rRNA gene and differed in only 5-8 out of 1802 bp. The non-photosynthetic *P.rotundatum,* however, differed in ca. 60 bp compared to the three photosynthetic species. This supports the original distinction between *Dinophysis* and *Phalacroma*. In the phylogenetic analyses the *Dinophysis/ Phalacroma* (dinophysoid) species fall into a common clade that are associated to a branch composed of gymnodinioid, prorocentroid and peridinioid species (GPP complex). Despite differences in morphology between the five isolates of *D. acuminata* , they all had almost identical ITS1 sequences. Similarly, all 5 isolates of *D.norvegica* were identical in this non-coding region. The ITS1-sequences in *D. acuminata* and D. norvegica were very similar, differing in << 10 bp. Further work includes development of oligonucleotide probes based on sequence differences within the rRNA operon.

ECOSYSTEM EFFECTS OF *PHAEOCYSTIS POUCHETII* AND ITS TOXINS

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Earlier research have shown that the haptophycean *Phaeocystis pouchetii* is toxic and that the excretion of toxins takes place at all phases of vegetative growth. There is thus a slow buildup of toxins over time in surface waters during a *Phaeocystis* bloom. Illustrating this by using examples from field situations and experiments we show that there are, from a ecosystem point of view, three phases in a *Phaeocystis* bloom: I. The coexistence phase where other phytoplankters and animals can share the biota with, but is influenced by *Phaeocystis* toxins; II. The mortality phase where all other species die out and/or escape and III. The "blank water" phase where plant and animal biomass approaches zero. Several of the effects of the toxin in question have consequences for commercial fisheries. The toxin may influence the feeding behaviour of cod larvae, or they may even experience mass mortality, food intake in aquacultured cage kept fishes may be reduced during spring, and we also claim that the cod spring fisheries along the coast of North Norway is brought to an end because buildup of toxins in the surface waters forces the fishes to migrate to deeper outlying waters. The scenarios we sketc illustrate that *Phaeocystis* is probably the single most important species in northern temperate areas that influences all trophic levels. It is food for herbivorous copepods and fish larvae, but it can also act harmful during periods of toxin buildup. Interesting is also, taken this in view, that *Phaeocystis* abundance varies between years, and that toxin production per unit *Phaeocystis* biomass increases with irradiance. By simple model studies we illustrate how variations in initial stock size, irradiance and dispersion rate may produce large between years effects on the ecosystem inhabitants.

ON IDENTIFICATION AND CLASSIFICATION OF *PROROCENTRUM*-SPECIES (PROROCENTRALES, DINOPHYCEAE) WITH SPECIAL EMPHASIS ON TOXIC SPECIES.

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More than 100 species have been described among the genera *Prorocentrum* Ehrenberg, *Exuviaella* Cienkovsky , *Dinopyxis* Stein, *Postprorocentrum* Gourret and *Mesoporos* Lillick belonging to the order Prorocentrales. At least 5 species are producing ocadaic acid and/or other DSP-toxins. Identification of the genus *Mesoporos* is easy and will be excluded from our analyses. In the last review of the genus *Prorocentrum*, Dodge (1975) regarded *Exuviaella, Dinopyxis* and *Postprorocentrum* as later taxonomic synonym of *Prorocentrum.* Dodge recognized only 21 species. In the last few years several new species, in particular benthic, potentially toxic ones, have been described. New features for separating species have been introduced, including molecular biological characters and the genus *Exuviaella* has been reinstated. Therefore a revision of the Prorocentrales is urgently needed. We correlated morphological and cytological characteres now used for delimitation of the species with molecular genetic analyses. The species investigated, including some so far undescribed ones fall into 2 major clades which do not correspond to morphological and/or ultrastructural distinct groups. Each of these clades can be further subdivided into 3 smaller clades that correspond to species clusters. We do not accept the reinstatement of the genus *Exuviaella* and regard a splitting of the genus *Prorocentrum* into different genera as premature given the present state of knowledge.

A PARASITE OF *ALEXANDRIUM MINUTUM* IN FRENCH COASTAL WATERS

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Parasites have been observed to affect all major algal classes, but few detailed reports of parasitic infection of dinoflagellates exist. By causing lysis of host cells, parasites may influence the population of the host. As an example, many *Alexandrium minutum* cells have been found to be infected by an unknown parasite after a bloom in the estuaries of northern Brittany, France. The new parasite is probably similar to the new genus *Parvilucifera* belonging in the Apicomplexan complex, recently described from scandinavian waters. The release of the biflagellate zoospores was video recorded under light microscopy, and the sporocyst structure was examined with the scanning electron microscope. Direct preparations of the zoospores, examined with the transmission electron microscope, show typical hair ornamentation on the long flagellum, the short one being naked and acronemated. The parasite was found to infect laboratory cultures of several other dinoflagellate species but the contamination appeared to be restricted to this taxonomic group as diatoms and a raphidophyte strain were not infected. Estimates of parasite-induced mortality indicate that it is capable of removing a significant fraction of dinoflagellate biomass in a short time. The same parasite was found again in natural samples during summer 1999, but the effect of this parasite on natural populations remains to be estimated.

TOXIC CYANOBACTERIAL BLOOM PROBLEMS IN AUSTRALIAN WATERS: RISKS AND IMPACTS ON HUMAN AND ANIMAL HEALTH

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The people of Australia are very familiar with toxic cyanobacterial blooms, as they have been a long-standing problem for agricultural and human drinking water supply, as well as for the recreational use of water. Livestock poisoning by cyanobacteria was first described in the last century near Adelaide, and the names of water-courses such as 'Poison Waterhole Creek' across the country reflect the hazard from cyanobacteria. More recently 1,000km of the Darling River carried a massive bloom of PSP containing *Anabaena* which killed an estimated 10.000 livestock and required emergency water supplies for several towns.This year (and last) in the centre of the City of Adelaide the Torrens Lake (no longer used for water supply) had a heavy bloom of toxic *Microcystis*, with waterfowl deaths. While livestock poisoning is relatively common,cases of human and wildlife poisoning are however rare, more through avoiding drinking evil-smelling water than to an absence of toxicity in cyanobacterial blooms.Effective assessment of the risk to human health requires data which relate the dose of toxin to the clinical effects in a population. When ,in the past, an adverse health effect from a cyanobacterial bloom has been observed in the population, no measurements of toxin in the water supply have been made. Even in the recent case of deaths of 50 dialysis patients in Brazil ,the best that could be achieved was retrospective analysis of post-mortem samples for toxin. As a result animal toxicity data are used for risk assessment, incorporating safety factors, to derive Guideline Values for a safe water supply.WHO has just announced the first of these values for cyanobacterial toxins, for the toxin microcystin,of 1.0 μg/litre of water.The other major potential hazards in water supplies in Australia are cylindrospermopsin from the tropical *Cylindrospermopsis*, and PSPs from *Anabaena.* All three toxic cyanobacteria occur in water supply reservoirs as intermittent blooms, often controlled by the supply authority by copper sulphate application.This lyses the bloom liberating toxins free into the water.Children tend to be more vulnerable than adults to toxins in the drinking water, partly because they have less choice of what they drink.Blooms usually occur in summer,when water consumption is high and swimming is popular.These hazards are recognised,bloom warnings are given on the radio and by erection of signs by rivers and lakes.Monitoring for blooms in reservoirs and rivers is essential to reduce the risks to the population and is undertaked widely in Australia.

IDENTITY OF BACTERIA ISOLATED FROM THE DOMOIC-ACID-PRODUCING DIATOM *PSEUDO-NITZSCHIA* MULTISERIES AND INVESTIGATIONS INTO MECHANISMS BY WHICH THEY INFLUENCE TOXIN PRODUCTION

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Domoic acid (DA) is the principle compound associated with the toxic syndrome Amnesic Shellfish Poisoning (ASP) and is produced by several species of the pennate diatom genus *Pseudo-nitzschia*, including *P. multiseries*. Previous studies with axenic cultures of this diatom demonstrated that DA was produced in the absence of bacteria, although the concentration was vastly reduced. Re-introduction of bacteria to the axenic diatom culture resulted in a 2 to115-fold increase in DA production, depending on the *Pseudo-nitzschia* culture and the bacterial strain. The mechanism by which bacteria cause this effect has not been elucidated. Here, the bacterial population associated with *P. multiseries* cultures will be described as will studies investigating the ability of these bacteria to produce DA autonomously. The possible role of signalling chemicals, such as homoserine lactones, will be discussed and also studies on DA metabolism by bacteria.

THE OCCURRENCE OF AMNESIC SHELLFISH POISONS IN SCOTTISH WATERS

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Monitoring for amnesic shellfish poisons (ASP) in wild and cultivated molluscs from Scottish waters commenced in 1998, following the of introduction of revised legislation from the European Community. The principle toxic compound, domoic acid (DA), was detected in a range of species and in some instances at concentrations above the regulatory limit. Although some harvesting closures were implemented in 1998, ASP caused much greater problems the following year. In 1999, the majority of scallop *(Pecten maximus)* fisheries from the Scottish west coast were closed due to ASP, resulting in considerable media attention and speculation on the role of nutrient inputs from local aquaculture. This paper will present data summarising the principal features of the 1999 toxic event. Although it is not possible to unambiguously identify the cause of such an event, the aim of this paper is to objectively summarise available information relating to the 1999 Scottish west coast outbreak, and to document the event as fully as possible.

PRELIMINARY ASSESSMENT OF THE EFFECTS OF CLIMATE CHANGE ON RISKS FROM CYANOBACTERIAL BLOOMS

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Anecdotal evidence suggests toxin producing cyanobacteria are becoming more abundant in drinking water reservoirs around the world. In Queensland, the three most common toxin producing cyanobacteria found in freshwater storages are *Microcystis aeruginosa, Anabaena circinalis* and *Cylindrospermopsis raciborskii.* Blooms of these species have public health implications due to their known potential for the release of hepatotoxins (*M. aeruginosa* and *C. raciborskii),* and neurotoxins (*A. circinalis*). This paper will present initial results from a study which aims to predict the effects of climatic changes on the occurrence and distribution of these potentially harmful cyanobacteria. The results from examination of historical limnological data will be used and integrated with predictive climate models to assess potential future risk from cyanobacterial blooms. The application of an Artificial Neural Network as a modelling and predictive tool of cyanobacterial blooms in reservoirs will be examined. Such systems have proved useful in the past for prediction of bloom events, which allows time for the implementation of strategies to reduce the impact of cyanobacterial toxins on public water supplies. The information from the modelling approaches has provided the framework for experimental investigations incorporating mesocosm and laboratory culture experiments.

DETECTION OF NSP (BREVETOXINS) IN ALGAL CULTURES AND SEAWATER BY ELISA

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We have raised antibodies against brevetoxin (PbTx-3), and established a direct competitive ELISA suitable for use on shellfish and seawater containing marine algae. The ELISA has a limit of quantitation of 0.53 ng /ml, and limit of detection below 0.1 ng/ml. The high sensitivity of the ELISA permits use with algal cultures and the direct analysis of seawater. The ELISA has great potential for monitoring bloom dynamics and for identification of toxic dinoflagellates. Extracts of cellular material from cultured algae were prepared by centrifugation, followed by extraction of the cell pellet with methanol. These extracts were diluted for ELISA. Brevetoxin was detected in a number of isolates. Medium was also harvested from *Gymnodinium breve* cultures by aspiration, and gentle centrifugation to remove the cells. An aliquot of medium was analysed by ELISA. Brevetoxin was detected at relatively high levels in the culture medium of a number of high producing isolates (no less than 20% of the toxin detectable in the pellet on a 'per cell' basis). The detection of brevetoxin at relatively high levels in the culture medium was rather unexpected. The ability to detect brevetoxin in cultured isolates opens the door to many areas of research, the most immediate benefit is the potential to characterise *Gymnodinium* species, and perform a chemo-taxonomic study. Of interest to regulators will be the ability to screen a wide range of algal cultures and taxa for the detection of toxin producing species. A number of the incidents of NSP toxin related closures in New Zealand have occurred in areas where *Gymnodinium* species were not detected.

A FAST FLUORIMETRIC ASSAY (FFA) FOR THE DETECTION OF SAXITOXIN IN NATURAL PHYTOPLANKTON SAMPLES

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A fast fluorimetric assay (FFA) for the detection of saxitoxin and its congeners in plankton samples is presented and is compared with the analysis by HPLC. The correlation between the results of the assay and those of the HPLC is significant for most of the carbamoyl saxitoxins. In culture experiments, saxitoxin producing dinoflagellates could be clearly distinguished from those which do not produce this toxin by use of FFA. During research cruises to the Orkney Islands and the Firth of Forth (Scotland) in 1997 and 1998, samples of toxic *Alexandrium tamarense* blooms were analysed by FFA. In 1998 detailed vertical profiles from the surface to 40 m water depth were sampled during two drifting experiments east of the Firth of Forth and east of the Orkney islands. We were able to show that saxitoxin fluorescence was found predominantly in the upper water layer and was not correlated with chlorophyll a concentration as measured by the Chlorophyll sensor of the CTD probe. The fast fluorimetric assay is easy to use and can be a helpful tool for ecological and also monitoring tasks concerning the detection of saxitoxin producing dinoflagellates.

A NEW SPECIES OF TOXIC *PFIESTERIA*

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We describe a second ichthyotoxic species within the genus *Pfiesteria* from the Albemarle-Pamlico and Chesapeake Estuaries of the eastern U.S., and will announce its soon-to-be-formal name in honor of a scientist who has contributed greatly to toxic dinoflagellate research. The second known toxic *Pfiesteria* species is polymorphic with a complex life cycle that includes an array of flagellated, amoeboid, and cyst stages. Its life cycle and behavior are, thus far, identical to that of *Pfiesteria piscicida*. This heterotrophic species can become mixotrophic with kleptochloroplasts, and it includes toxic, temporarily nontoxic, and never-toxic strains. The mesokaryotic zoospores (7-12 μm) contain thin thecal plates arranged in a Kofoidian series of Po, cp, X, 4', la, 6", 6c, 4s, 5'", 2"". Their cysts are chrysophyte-like with organic scales and bracts; and they can also form temporary mucoid cysts. The eukaryotic, benthic amoeboid stages are morphologically filose to lobose, with reticulated cysts and length ranging thus far from 5 to 380 μm. Anisogamous gametes fuse to form planozygotes with one transverse and two longitudinal flagella, which can become hypnozygotes. This species is distinguishable from *P. piscicida* both morphologically and genetically. Like *P. piscicida*, it shows strong preference for certain species of algal prey when fish are not available, and its toxicity varies depending on its history of access to fish prey.

WORLDWIDE OCCURRENCE OF PECTENOTOXINS AND YESSOTOXINS IN SHELLFISH AND PHYTOPLANKTON

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Pectenotoxin-2 and its seco acid were identified for the first time from Chile in two species of mussels and in *Dinophysis norvegica* collected along Swedish coasts (Baltic Sea and west coast). In *D. norvegica* from Baltic Sea, dinophysistoxin-1 and okadaic acid were detectable only after hydrolysis of extracts. The Chilean mussels contained high concentrations of yessotoxin (YTX) and 45-hysdroxy-YTX. YTX and pectenotoxin-6 were the major causes for closure of scallop farms in Mutsu Bay, Japan. Clearly we need to pay more attention to toxins other than okadaic acids and apply proper regulation levels for individual toxin groups.

THE POTENTIAL FOR THE INTRODUCTION OF DINOFLAGELLATE CYSTS INTO THE PORTS OF ENGLAND AND WALES

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Ships' ballast tanks may contain a range of viable organisms in both the water and sediment. This report examines dinoflagellate cyst populations in a total of 113 sediment samples, 96 samples of which were collected from vessels discharging ballast water and the remainder from vessels in dry dock. Full cysts, identifiable to at least genus level, were found in 69% of samples. A total of 49 species were identified, representing 20 genera. *Pentapharsodinium dalei* and *Scrippsiella trochoidea* occurred in 23 and 22% of samples respectively. A maximum of 22 cyst types were found in a single sample, with a mean of <5. The highest cyst density recorded was 8950 cysts per ml of wet sediment but the majority of samples contained < 400 cysts per ml. However, numbers of cysts recorded varied with the sampling method and there were further significant differences in total cyst concentrations and species diversity between tanks on a single ship. No relationship between residence time of sediment and cyst numbers was found. In addition to the hatching of observed cysts, slurry enrichments also produced motile stages of smaller species unrecorded visually in surveys, indicating cyst isolation underestimates total species composition. Most cyst types found in this study have been recorded from U.K. waters. Exceptions were *Scrippsiella hangoei*, a bloom forming species recorded in 9 samples, while *Gymnodinium nolleri, Pentapharsodinium tyrrhenicum*, *Pyrophacus steini* var. *vancampoae* and *Alexandrium* species, including *A. minutum* and *A.* c.f. *leei*, were also found. Representatives of this genus, which contains many toxic species, were recorded in 28 (25%) samples. Of five types recognised, *A. tamarense / catenella* were most common, occurring in 19 (17%) samples. This study is a confirmation of observations made elsewhere, demonstrates the ubiquity of viable cysts, and shows that amongst these will be cysts of potentially toxic species.

EVIDENCE FOR A NEW GENUS WITHIN THE *GYMNODINIALES* FROM DIFFERENT DATASETS

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Different datasets are used in systematics to help identify homologous characters and natural groups among similar organisms. Morphological, ultrastructural, thecal polysaccharide, pigment, toxin, and molecular analyses of the international reference species *G.mikimotoi, G.breve*, and both similar and morphologically different species from New Zealand were compared. The total evidence and congruence among morphological and biochemical characters strongly suggests that a suite of species currently assigned to either *Gymnodinium* or *Gyrodinium* are closely related to each other and not to the type species *Gymnodinium* fuscum or *Gyrodinium spirale*. A summary of this work will be presented with detailed inter-laboratory studies of pigment and toxin analyses presented separately.

IMMUNOCHEMICAL LOCALIZATION OF MICROCYSTIN-LR AND ASSOCIATED PATHOLOGICAL CHANGES IN RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*

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Intoxications with microcystins have lead to death of humans and animals such as livestock or fish. In order to determine the localization of microcystin in trout, an antibody was developed against MC-LR and tested for suitability of toxin detection in liver and other tissues of gavaged trout and in isolated primary trout hepatocytes. Polyclonal as well as monoclonal antibodies against microcystin-LR were produced and used for microcystin detection in tissue homogenates or in tissue sections. Microcystin was probed in tissue, cell and subcellular homogenates after in vivo and in vitro exposure to the toxin by immunoblotting and immunocytochemistry. In order to identify putative protein adducts, Western blots were also stained with anti-human protein phosphatase 2A (PP2A). Organ pathology was described in hematoxylin & eosin stains. The results show that the antibodies can detect microcystin-LR in trout organs and hepatocyte homogenates. Several microcystin-protein-adducts with a molecular weight of approximately 35 kD could be characterized. One of these adducts was identified as PP2A. Immunoblotting of hepatocyte proteins showed that the activity was largely localized in the cytosol, while immunocytochemistry demonstrated cytosolic as well as nuclear staining. Immunohistochemistry revealed a time dependent discernible increase in staining intensity throughout the liver, concurring with the kinetics of hepatic PP-inhibition. Organ pathology showed hepatocyte necrosis as an early event with secondary apoptotic changes after 48 hours at the earliest. This study suggests that accumulation of MC and subsequently changes in cellular morphology, PP-inhibition and hepatocyte necrosis represent the primary events in microcystin induced hepatotoxicity, while apoptotic cell death may be only secondary.

EFFECT OF OZONATION IN DRINKING WATER TREATMENT ON THE REMOVAL OF CYANOBACTERIAL TOXINS AND TOXICITY OF BY-PRODUCTS AFTER OZONATION OF MICROCYSTIN-LR

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The presence of cyanobacterial toxins such as microcystin in drinking water supplies poses a serious health risk to humans and may result in chronic liver injury and possibly in the promotion of liver tumors. It is thus important to monitor cyanobacterial densities and toxin levels in water reservoirs and, in the event of a bloom, to remove these toxins by adequate water treatment procedures. Conventional water treatment is ineffective in reducing cyanobacterial toxin levels to below acutely toxic concentrations. Previous studies have suggested that the best method to remove cyanobacterial toxins from drinking water is oxidation with ozone. In order to investigate the efficacy of ozone in the removal of cyanobacterial toxins, *Microcystis aeruginosa* PCC 7806 and *Oscillatoria rubescens* from Lake Zurich, Switzerland were ozonated in a batch reactor with O_3 concentrations ranging from 0,3-2 mg/l for 9 min contact time and 60 min reaction time (ozone off). The presence of toxins was detected by a protein phosphatase inhibition (PPI) assay, HPLC, ELISA, immunoblotting and a primary hepatocyte toxicity assay. Products of ozonation obtained following incomplete oxidation of microcystins were analyzed by HPLC, ELISA, PPI and immunoblotting. The results show that the residual toxicity of the cyanobacterial material depended on the cell density, the ozone concentration, the duration of ozonation and the temperature of the ozonated water. Complete detoxification of 10^5 cells/ml was achieved with 1.0 mg O_3/l. Ozonation of higher cell densities resulted in increased toxicity due to lysis of cyanobacterial cells and release of toxins. A residual level of ozone (0,05 mg/l) should therefore remain in order to completely destroy the toxins. The importance of this was shown by the observation that ozonation products may display PPI and were detectable by ELISA and immunoblotting.

THE EFFECT OF THE DAWESVILLE CHANNEL ON CYANOBACTERIAL BLOOMS AND ASSOCIATED PHYTOPLANKTON IN THE EUTROPHIC PEEL-HARVEY ESTUARY AND ITS ASSOCIATED WATERWAYS MANDURAH, WESTERN AUSTRALIA.

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The construction and opening in April 1994 of the 200 metre wide Dawesville Channel in April 1994 connecting the Peel-Harvey estuary at Mandurah in Western Australia, to the Indian Ocean, has had an impact on the integrated phytoplankton composition and density of the eutrophic waterway Peel-Harvey Estuary. The Channel. connects the Harvey Estuary at Mandurah in Western Australia, to the Indian Ocean. The almost annual spring-summer blooms (max. ca. 600,000 cells per mL) of the hepatotoxic cyanobacterium *Nodularia spumigena* Mertens that occurred in the eutrophic estuary between 1978 and 1992, have ceased to occur since the Channel was opened to the sea. Though the Estuary still experiences occasional phytoplankton blooms these are dominated mainly by estuarine and marine species of diatoms. There have been rare isolations of *Nodularia* in the estuary Estuary and these have been attributed to material brought in from estuarine reaches of the Serpentine River portions of rivers. Annual blooms of *Nodularia* still continue to occur in the Serpentine River estuary this river.

TOTAL PRODUCTION OF C1 /C2 BY *ALEXANDRIUM TAMARENSE*

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Sufficient amounts of C1/C2 and GTX2/3 have been produced for metabolism studies from a Hong Kong strain of *Alexandrium tamarense* that produces C1/C2 in high yields. The toxic dinoflagellate was cultured in Fernbach flasks in artificial seawater K-medium at 23C with 5000 lux and a 16/8 hour light cycle. Our results indicate that large quantities of C-toxins are present extracellularly in the culture medium of batch cultures of *Alexandrium tamarense*. Intracellular toxins were extracted by sonication of harvested cells. Extracellular toxins were extracted and concentrated by passing the spent culture medium through a strong anion exchange column. Toxicity throughout the study was followed using the mouse neuroblastoma assay. Toxin content was analyzed using HPLC-FLD. Intracellular concentrations of C1/C2 were found to be approximately 16 fmol/cell at late log phase with a comparable proportion of toxins present in the culture medium. In this case, recovery of intracellular toxins alone would underestimate the total toxin production by up to 50%. Combining both toxin sources, in which one half of the C-toxins were hydrolyzed under optimized acidic conditions to GTX2/GTX3, led to the recovery of mg quantities of purified toxins for further studies. These findings indicate that: 1) spent culture medium is a readily available source of toxins and 2) toxins present in the culture medium should not be ignored in determining the total toxin production by *Alexandrium*.

RELATIONSHIP BETWEEN THE POPULATION DYNAMICS OF *CHATTONELLA* SPP. (RAPHIDOPHYCEAE) AND THE ALGICIDAL BACTERIUM *CYTOPHAGA* SP. IN THE SETO INLAND SEA, JAPAN

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A marine algicidal gliding bacterium *Cytophaga* sp. strain J18/M01 was isolated using the harmful red tide alga *Chattonella antiqua* as a susceptible organism from a station in northern Harima-Nada, the Seto Inland Sea, Japan, in 1990. The bacterium can prey upon various species of microalgae. Temporal fluctuations of this bacterium and *Chattonella* spp. (*C. antiqua* and *C. marina*) were investigated weekly at the above stationin the summer of 1997 and 1998, with the immunofluorescent assay employing polyclonal antibodies for the bacterium *Cytophaga* sp. strain J18/M01. This polyclonal antibody showed the highly specific reactivity, and other several phylogenetically close bacteria did not react with this antibody. In the summer of 1997, the cell density of *Chattonella* spp. showed a maximum value (70 cells / ml) on 8 July, and decreased thereafter. The bacterium *Cytophaga* sp. was commonly detected a round a few hundreds cells / ml or less. The number of *Cytophaga* sp. increased after the peak of *Chattonella* spp. and the maximum cell number of the bacterium was 1300 / ml. This algicidal bacterium also followed the changes of total amounts of microalgal biomass (chlorophyll-a + pheophytin) when *Chattonella* spp. were absent. In the summer of 1998, *Chattonella* spp. were scarce, and the algicidal bacterium *Cytophaga* sp. showed a close relationship with the change of total microalgal biomass. The present results suggested that the algicidal bacterium *Cytophaga* sp. preyed upon not only harmful red tide microalgae but also common microalgae such as diatoms when they are blooming, and the bacterium presumably play an important role in regulating microalgal biomass within some homoeostatic level in the coastal sea.

OCCURRENCE AND SUCCESSION OF POTENTIALLY HARMFUL PHYTOPLANKTON SPECIES IN THE EASTERN HARBOUR OF ALEXANDRIA

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Since first observed in 1956 the red tide blooms of *Alexandrium minutum* Halim remained a recurrent summer phenomenon in the type locality of the species, the eutrophic Eastern Harbour of Alexandria. The year 1994,however, has seen its last red tide, followed by the complete disappearence of the species. Daily observations in the summer of 1999 showed *A. minutum* to have been replaced by a community of potentially harmful species: *Pseudonitzschia pungens, Prorocentrum minimum, P. triestinum* and others. Alternate dominance of this community with diatoms, cyanophytes and euglenophytes is modulated by and significantly correlated to the wide salinity fluctuations, the wind velocity and the stability of the water column. The amplitude and duration of the bloom pulses is controlled by the grazing pressure of tintinnids, copepods and planktonic larvae.

GERMINATION CHARACTERISTICS OF NATURAL OCCURRING CYSTS OF *ALEXANDRIUM TAMARENSE* IN HIROSHIMA BAY, INLAND SEA OF JAPAN

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In order to examine temporal changes in germination ability, time to germination and auto-fluorescence property of the resting cysts of *Alexandrium tamarense*, long-term investigation was conducted in Hiroshima Bay, where spring bloom (March to May) of *A. tamarense* has been observed almost every year since 1992. Approximately fifty cysts were isolated monthly from the bottom sediment between June 1994 and June 1997. The cysts were incubated on the day of sampling under in situ bottom water temperature condition, and germination success and emission of auto-fluorescence were checked every day. High germination success rates (> 50 %) were observed between December and April every year (bottom water temperature = 10.0 to 16.5 C, with an average germination time of 10.2 days (n = 455). On these occasions, cysts started to emit red auto-fluorescence few days before the germination and no obvious temporal change in germination time was observed. From June to November, germination success rates were considerably low (0 to 40 %, bottom water temperature = 14.6 to 25.1 C). Particularly, no germination was observed in September (bottom temperature = 23.6 to 25.1 C). Relationship between the incubation temperature and the rate of germination success suggested that the cysts have a "temperature window" (ca. 10 to 15 C) for germination, which would be responsible for the seasonal changes in germination ability. The present results indicate that the germination characteristics of *A. tamarense* cysts are well adapted to the ambient water temperature rhythm in the temperate shallow coastal environment, allowing *A. tamarense* to seed vegetative cell population for the spring bloom.

AZASPIRACID POISONING (AZP): A NEW SHELLFISH TOXIC SYNDROME IN EUROPE

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Human intoxications, following the consumption of Irish mussels (M. edulis), have occurred in The Netherlands (1995), Arranmore Island (1997), France (1998) and Italy (1998). The contaminated shellfish were cultivated in four different regions encompassing the entire west coast of Ireland. These intoxications have now all been attributed to a new family of shellfish toxins. Three toxins have been isolated and structurally elucidated and they contain a novel spiro ring assembly. The major toxin, has been named azaspiracid (AZ-1) but substantial amounts of its methyl and demethyl analogues, AZ-2 and AZ-3, respectively, were also present in shellfish. The aetiology of azaspiracids and toxin dynamics in shellfish have been studied using liquid chromatography-mass spectrometry methods, especially LC-MS, and strong evidence has been obtained that azaspiracids are produced by a dinoflagellate. Azaspiracids are persistent in shellfish and have been found in mussels as long as eight months after the initial intoxication. In several instances, toxin levels in oysters (*C. gigas*) have been comparable to the levels found in mussels from the same cultivation area. These toxins have also recently been identified in mussels from England and Norway which implies a more widespread intoxication of European shellfish than has previously been appreciated. In animal tests, azaspiracids show pronounced neurotoxic effects and produce severe damage to the intestine, spleen and liver tissues. Since azaspiracids are toxicologically and chemically different from previously identified groups of shellfish toxins, a new toxic syndrome has been declared and named, azaspiracid poisoning (AZP).

COMPARISON TRIAL OF A RAPID TEST FOR PARALYTIC SHELLFISH POISONING (PSP) AND THE AOAC MOUSE BIOASSAY

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Jellett Biotek Ltd. has developed a rapid test to screen for paralytic shellfish poisoning. The new test, called MIST Alert for PSP provides a qualitative (yes/no) indication of toxicity in less than 20 minutes. The test is designed as a screening tool for PSP in regulatory labs by screening out negative samples leaving a small number of positive samples to be tested with more expensive analytical tests. Due to the simplicity of the test and its rapid response, it can also be used in harvest management applications to indicate when shellfish are safe to harvest or as a quality control tool in a shellfish processing plant.

The MIST Alert technology was tested using pure standards including neosaxitoxin, (NEO), saxitoxin (STX), decarbamoyl saxitoxin (dcSTX) and gonyautoxin 2/3 (GTX2/3) from the certified reference materials program (PSP-1B) of the National Research Council of Canada. Limits of detection of pure toxins diluted with running buffer were found to be 35,20, 35 and 30 ng/ml respectively, compared to the regulatory limit for closure of 400 ng/ml (equivalent to 80μg/100g) used in most countries.

Over 500 shellfish extracts (AOAC extraction method for PSP) from a previous parallel trial of the MIST Quanti cell based kits at the Department of Environmental Conservation Lab (DEC) in Palmer, Alaska, were tested on the MIST Alert for PSP, and the results for each sample compared to the results previously obtained by DEC using the AOAC mouse bioassay. The extracts were taken from diverse geographical areas and represented diverse shellfish tissue type and a broad range of toxicity.

Results of parallel testing of the Alaskan samples and applications of the technology for the regulatory environment and aquaculture industry will be discussed.

POTENTIALLY CHEAP MITIGATION OF RHEOTOXICITY, CYTOTOXICITY AND FISH MORTALITY CAUSED BY THE DINOFLAGELLATES, *GYMNODINIUM MIKIMOTOI* AND *G.* CF. *MAGUELONNENSE*

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We investigated different compounds for activity in mitigating the lethal effects on fish of two dinoflagellates, *Gymnodinium mikimotoi* and the morphologically similar *G.* cf. *maguelonnense.* Measurements were made of survival of seabass in cultures of both species of dinoflagellate, flow of the cultures through the channels between their gills, and on haemolytic activity of the cultures. Both dinoflagellates showed a strong tendency to kill fish. *G. mikimotoi* at 23,000 cells/ml reduced the survival time of fish 40%, but showed no detectable haemolytic activity. *G.* cf. *maguelonnense* at 3000 cells/ml, however, reduced survival time by 74% and showed high haemolytic activity. *G. mikimotoi* showed more rheological acitivity, while *G.* cf. *maguelonnense* was the more haemolytic. Two compounds were found to mitigate the rheological and haemolytic activities of these two dinoflagellates, as well as their tendency to kill fish. These compounds were N-acetyl-L-cysteine and ethyl-L-cysteine ester. The optimal concentration of both was around 0.1 mM (~155 g/m^3). Each compound therefore reduced the fish-killing tendencies of both dinoflagellates. By treating water containing fish stocked or concentrated at suitable density (~4 kg/m^3) in a fish farm, the cost per treatment would be 0.3 to 3 US cents/tonne of fish.

INCREASE IN THE PRODUCTION OF ALLELOPATHIC SUBSTANCES BY *PRYMNESIUM PARVUM* CELLS GROWN UNDER N OR P DEFICIENT CONDITIONS

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The marine haptophyte *Prymnesium parvum* is known to produce a set of highly potent exotoxins commonly called prymnesins. These toxins have been shown to have several biological activities, including ichthyotoxic, neurotoxic, cytotoxic, hepatotoxic and hemolytic activity towards a range of marine organisms, mainly gill breathing animals such as fish, shellfish and molluscs. If prymnesins can act as allelopathic substances inhibiting the grouth of other phytoplankton species is however not known. Earlier studies have shown that the toxicity of *P. parvum* is enhanced when the cells are grown under N- or P-limited conditions. In this study, *Thalassiosira weissflogii, Prorocentrum minimum, Rhodomonas* sp. and a strain of *Prymnesium patelliferum* known to produce prymnesins, were incubated with cell-free filtrate of *P. parvum* cultures grown under nutrient limiting (N or P) or non limiting conditions. Addition of filtrate from *P. parvum* cultures grown under N- or P-limitation inhibited the growth of all tested species, except *P. patelliferum,* which was not negatively affected under any conditions. Also, addition of filtrates from the non-limited cultures did not have a negative influence on the growth of any of the tested species. This differing activity of the *Prymnesium* toxins suggests that prymnesins may play an allelopathic role in nature. Thus, production of prymnesins is a valuable weapon in *Prymnesium*- species to avoid grazing pressure and to outcompete co-occuring phytoplankton species.

CYANOBACTERIAL BLOOM ECOLOGY AND MANAGEMENT. THE AUSTRALIAN EXPERIENCE

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From late spring through to autumn cyanobacterial blooms are a common feature of reservoirs and river weir pools throughout Australia. While usually not large in comparison with eutrophied European water bodies – biomass peaks of 10-30 μg chl a L-1 are typical – these blooms are wide spread; from the tropical and sub-tropical zones in the north of the continent to the warm temperate and arid regions in the south. The moderate size of the blooms reflects the fact that anthropogenic nutrient inputs to waterways are lower than in other parts of the world where human population densities far exceed those of inland Australia. As in the cooler parts of the world, the dominance of the freshwater phytoplankton by cyanobacteria for several weeks or months each year is related to the onset and persistence of thermal stratification. In Australia's hot arid climate the period of stable stratification in reservoirs and slow-flowing rivers may last for 9-10 months of the year. Combined with the high net solar insolation, this provides conditions that are ideal for buoyant cyanobacteria. This selective pressure is further enhanced in our clay-turbid rivers where photic zone depths are often less than 1-2 metres. Bloom frequency also shows a climate related inter-annual variability coupled to variations in the El Nino-Southern Oscillation (ENSO). Other factors, particularly those relating to reservoir food web structure and imbalances, may also contribute to the dominance of cyanobacteria. Recent work by CSIRO has shown that the excessive populations of planktivorous fish found in Australian reservoirs may stimulate a high phytoplankton biomass and favour cyanobacteria over other phytoplankton. The management of cyanobacterial blooms in Australia has developed rapidly during the past decade. There has been a broadening of the managerial focus from a single-pronged strategy addressing only the control of catchment nutrient inputs, to a strategy which combines catchment management with in-reservoir techniques such as destratification, sediment remediation, biomanipulation, and flow-regulation of rivers. Whether these approaches will ultimately be successful, or whether Australia's climate is too great an obstacle to cyanobacterial bloom management is yet to be seen. At the downstream, consumer end, management actions have focussed on refinements to drinking water treatment processes to ensure removal of cyanobacterial toxins.

ENVIRONMENTAL FACTORS AFFECTING THE NEUROTOXIN PRODUCTION OF *CHATTONELLA ANTIQUA* (RAPHIDOPHYCEAE)

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Neurotoxin production and ichthyotoxicity of *Chattonella antiqua* isolated from Yatsushiro Sea, Japan in 1978 were investigated at different temperatures and light intensities under laboratory conditions. Variation in temperature had a pronounced effect on toxin profiles of the flagellate. The yields of CaTx-II (corresponding to PbTx-2) and CaTx-III (corresponding to PbTx-3) peaked at 15 C with 0.39 and 3.18 pg/cell. As the temperature increased, the amount of CaTx-II and CaTx-III decreased gradually, but there was an increase in the amount of CaTx-IV (corresponding to oxidized PbTx-2). The concentration of CaTx-I (corresponding to PbTx-1) was high at 20 C (0.94 pg/cell). The sharp decrease in all fractions were found at temperatures above 25 C where the organism showed a little growth. Light intensity had a smaller influence on toxin profiles of this species. The yields of CaTx- I and III at 20 μE m-2 sec-1 were 0.86 and 1.84 pg/cell and decreased slightly with increased light intensity. The concentration of CaTx-II remained nearly constant between 20 and 100 μE m-2 sec-1 and lowered at high light intensities. Ichthyotoxicity showed the highest toxicity at 15 C and markedly reduced as the temperature exceeded 20 C. Cells cultured at 25 C were about 2 times less toxic than those cultured at 15 C.

GROWTH AND TOXICITY OF *NODULARIA* BLOOM IN THE WESTERN GULF OF FINLAND IN AUGUST 1999

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During August 2-12 1999, development of a surface bloom dominated by the cyanobacterium *Nodularia spumigena* was followed in the western Gulf of Finland, the Baltic Sea. Intense surface scums of aggregated *Nodularia* existed on calm days. The low-nutrient *Nodularia*-dominated water mass was separated by a front from the nutrient-rich *Aphanizomenon flos aquae*-dominated mass in the Gulf area. Due to inadequate mixing of the two water masses, it is likely that the *Nodularia* growth was based on the use of internal phosphorus storage (surface water phosphate concentrations between 0 and 0.05 μM). Signs of cell decay were observed on August 5 and the degree of empty filaments in the community grew higher towards the end of the survey period. A high number of the diatoms *Nitzschia* was recorded within the *Nodularia* aggregates on August 8, suggesting nutrient leakage from the *Nodularia* filaments. On-board, toxin analysis was carried out with HPLC and ELISA. The results from these tests indicated that decay of *Nodularia* or a decrease in the proportion of *Nodularia* cells in dry material resulted in decreasing concentrations of cell-bound nodularin (from 2.1 to 0.5 g kg-1 dw; HPLC). Results obtained with rapid ELISA kits correlated (r = 0.92, n=7) with HPLC results. On the average, ELISA provided 50% higher results than HPLC. No clear temporal trends in nodularin water concentration(<<0.5-2.6μg l^{-1}; ELISA) was observed. The bloom was probably linked to a fish kill during the same time. Dead three-spine sticklebacks (*Gasterosteus aculeatus*) were found floating on the surface. According to ELISA, the sticklebacks contained approximately 35-170 μg toxin kg-1 dw (MC-LR equivalents). Empty *Nodularia* cells and traces of nodularin were also found in surficial sediments collected from the study area which indicated that toxic *Nodularia* may reach the seafloor.

DEVELOPING A METHOD OF CONTROLLING THE OUTBREAK AND MAINTENANCE OF RED TIDES USING NAOCL PRODUCED BY ELECTROLYSIS OF NATURAL SEAWATER

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We investigated the possibility of using NaOCl produced by electrolysis of natural seawater to control the outbreak and maintenance of red tides. The densities of the red tide dinoflagellates *Cochlodinium polykrikoides, Gymnodinium sanguineum, Lingulodinium polyedrum, Gyrodinium impudicum, Scrippsiella trochoidea* rapidly reduced > the concentrations of 0.5 ppm in the laboratory experiments. However, the density of a naked ciliate *Strombidinopsis* sp. rapidly reduced the concentration of 1 ppm and that of a calanoid copepod *Acartia* sp. was not affected the concentration of 10 ppm. Half the concentration of NaOCl was converted to NaCl within 2 hours under bright sunlight. Therefore, NaOCl produced by electrolysis reduce the population sizes of red tide dinoflagellates with minimizing the secondary effects on the populations of ciliates and copepods at the concentrations of 0.5 ppm.

FROM THE HISTORY OF TOXIC ALGAL BLOOMS IN ISRAEL. THE CASE OF *PRYMNESIUM PARVUM* AND ITS CONTROL

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First noted in bloom condition in Israel during the late 40s, the haptophyte, euryhaline microflagellate *Prymnesium parvum* has been responsible, due to its production of an exotoxin, identified as prymnesin, for large-scale fish mortality, especially in brackish water ponds in the lower Jordan Valley and parts of the coastal plain. Its toxicity was found to be importantly dependent on pH and temperature. High cell levels of 5-8x10^5 cells/ml and more were not necessarily correlated with high toxin levels. Experiments to find an agent to control *Prymnesium* showed sensitivity of this organism to the ammonium ion. The common fertilizer component, ammonium sulphate, at a concentration of 1:100,000 caused *Prymnesium* to immediately swell, lose its flagella and subsequently lyse with generally none of the adverse effects on fish or other plankton, as can be seen with the algicide, copper sulphate, at a concentration of 2-3ppm. Its application, however, should be subject to continuous monitoring for the presence of *Prymnesium* due to ongoing nitrification processes, evaporation and the influx of fresh water into the ponds. Alternatively, liquid ammonia has been found to be a useful and inexpensive control agent at temperatures <20^{o}C and pH <8.5. Ponds in the same region as those with toxic *Prymnesium* but populated with N-fixing filamentous cyanobacteria such as *Anabaena* spp. in bloom condition have been found to be free of *Prymnesium*, thus suggesting a role for organisms capable of generating high levels of ammonium-N as natural shields against toxic blooms.

INTRACELLULAR BACTERIA IN THE BLOOM-FORMING DINOFLAGELLATE *NOCTILUCA SCINTILLANS*

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Although generally regarded as harmless in European waters, the bloom-forming dinoflagellate *Noctiluca scintillans* has been occasionally associated with fish mortality and reduced shrimp yields in Asia. The reason for this phenomenon is unclear, but it is possible that bacteria my be involved in harmful algal blooms; the bacteria may produce their own toxins or influence the toxic levels of the algae. It is thus noteworthy that up to 1 % of *Noctiluca* occurring in the southern North Sea contain large numbers of intracellular bacteria; these *Noctiluca* cells appear visibly turbid. Analysis of the diversity and dynamics of bacterial populations associated with *Noctiluca scintillans* by denaturing gradient gel electrophoresis (DGGE) indicates the occurrence of one dominant group of bacteria within *Noctiluca* and different other groups in smaller amounts. In contrast, free living bacterial populations in the water column consist of several different dominant groups.Comparative analysis of DGGE patterns of bacteria from turbid free living and laboratory cultured *Noctiluca* suggests that the dominant intracellular bacteria belong to the same groups, excluding a cultivation effect. Up to now 18 bacterial isolates from *Noctiluca* cells have been cultured. The bacteria have been characterized by classic physiological (including antibiotic sensitivity) and molecular biological methods. Phylogenetic analysis of the 16S rDNA of the bacteria revealed a great diversity among the bacterial isolates belonging to different groups of bacteria, i.e. bacteria of the g-subdivision of the Proteobacteria such as the *Xanthomonas* group, *Colwellia* assemblage, *Vibrio fischeri* assemblage, *Pseudoalteromonas* group, and of the Gram positive *Arthrobacter* group. The role and significance of the intracellular bacteria with regard to *Noctiluca* blooms is discussed.

KILLING OF A HARMFUL DINOFLAGELLATE *HETEROCAPSA CIRCULARISQUAMA*, WHICH CAUSES DAMAGES TO BIVALVES SUCH AS OYSTERS, BY A MARINE BACTERIUM EHK-1

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Red tides of a harmful dinoflagellate, *Heterocapsa circularisquama*, have caused mass mortality of bivalves such as oysters in Western Japan since 1988. For the purpose of microbial control of the red tide occurrence, *H. circularisquama*-killing microorganisms were screened. A marine bacterium EHK-1, which had a strong algicidal effect on *H. circularisquama*, was isolated from seawater of Etajima Bay, the Seto Inland Sea of Japan. EHK-1 was a gram-negative, long rod shaped bacterium that showed a gliding-like motility. Pylogenetic analysis of 16S rRNA gene sequences indicated EHK-1 was a novel marine bacterium belonging to proteobacteria gamma-subgroup. EHK-1 killed *H. circularisquama* within 24 hours when this bacterium was inoculated at the density of $1x10^4$ cells/ml to *H. circularisquama* culture in the exponential phase. During the killing process, both of the motility loss and the lysis of the microalga were observed. The filtrate from the co-culture of the EHK-1 and *H. circularisquama* was lethal to *H. circularisquama*. It suggests that EHK-1 killed *H. circularisquama* by means of extracellular substances.

ANTIBODY AGAINST PSP TOXINS RAISED BY NEWLY DESIGNED ANTIGEN

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PSP is one of the most important shellfish poisonings because of its acuteness of the symptoms, wide distribution, and high fatality. In the areas where shellfish are contaminated with PSP toxins, shellfish toxicities are monitored to avoid the poisoning. Currently , mouse bioassay is applied for detection of shellfish toxins in many countries. This is a simple and reliable method, but has many problems because of the animal assay. The cost of the assay also increases an economic loss of PSP problems. Therefore, a simple, rapid and cheap method for toxin detection is needed. For this purpose, ELISA is the first candidate. However, the antibody to detect various toxin components could hardly be obtained. Previously, we reported the formation of a stable conjugate, in which C11 atom of STXs and sulfur atom of thiols are covalently bound, in the course of the transformation of gonyauatoxins to saxitoxins by thiol compounds such as glutathione. In the present study, we tried to obtain the antibody using the antigen in which carrier protein was introduced to amino group of glutathione bound to C11 of saxitoxin. Although the activity to react with toxins was low, the antibody obtained reacted with all the toxin components examined almost equally.

PRODUCTION OF DOMOIC ACID AND MORPHOLOGY OF THE DIATOM *NITZSCHIA NAVIS-VARINGICA* SP. NOV., ISOLATED FROM A SHRIMP CULTURE POND IN DO SON, VIETNAM

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During a survey of algal species involved in mass mortalities of shrimp larvae in Asian countries, the neurotoxin domoic acid (DA) was detected by HPLC-fluorescence analysis in 5 monoclonal cultures of a non-*Pseudo-nitzschia* diatom isolated from a shrimp culture pond at Do Son, Vietnam in 1997. DA levels of the cultures ranged from 1.2 to 3.1 (average 2.3) pg/cell, which is within the range reported for *Pseudo-nitzschia multiseries*. Confirmation of DA was subsequently performed by ESI MS. As many *Pseudo-nitzschia* species, DA production of the cultures started during late exponential growth phase and reached a maximum during stationary growth phase, followed by gradual decrease. In contrast to *P. multiseries*, no significant difference in DA production was observed between axenic and non-axenic cultures. The diatom has been examined using light, transmission and scanning electron microscopy and is described as a new species, *Nitzschia navis-varingica* sp. nov. The genus *Nitzschia* is very large comprising more than 900 taxa, which taxonomically are collected into sections. *Nitzschia navis-varingica* fits best into a group of sections that includes Dubiae, Bilobatae, most of the Lanceolatae and Lineares, all sensu Grunow. Cells of the new species are lanceolate in valve view. The cells are slightly indented in the middle in girdle view, but not really bilobate as in the section Bilobatae. The interstriae are raised above the striae, each of which contains one row of areolae. The raphe is raised on a keel as in *Nitzschia* subgenus *Nitzschia,* but a conopea is absent (present in the type species of *Nitzschia*). *Nitzschia* species are found in both freshwater and marine environments and production of domoic acid may therefore be more widespread in the environment and among diatoms than presently thought.

OUTWARD EXCITOTOXIC EFFECTS AND TISSUE DISTRIBUTION OF DOMOIC ACID IN A PROMINENT VECTOR SPECIES, THE NORTHERN ANCHOVY (*ENGRAULIS MORDAX*)

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The planktivorous Northern anchovy (*Engraulis mordax)* is a prominent vector of the phycotoxin domoic acid (DA) to organisms at higher trophic levels, including fish-eating sea birds and mammals. Although there are abundant data reporting DA-induced neuroexcitotoxicity in these higher vertebrates, to date there has been no reported evidence of neurotoxic effects in lower vertebrate vectors such as fish. To explain this apparent lack of toxicity, it has been suggested that DA may not be absorbed from the digestive tract in fish and/or that fish are not as sensitive neurologically to DA. We have evidence that anchovies do absorb DA and that anchovies do have a similar neurologic sensitivity to DA as mammals. Anchovies collected from Monterey Bay California during a toxic bloom of *Pseudo-nitzschia australis* in May 1998 contained 223 +/- 5, 55 +/- 1, and 39 +/- 0.7 μg DA/g in viscera, whole anchovy, and body tissue samples, respectively. Inter peritoneal (IP) injection of DA at concentrations ranging from 5 – 11 μg DA/g in 20 anchovies (mean weight 20 g) resulted in individual circle swimming, spiral and upside-down swimming, surface circling with gaping mouth, complete inability to school, and death, while 20 control anchovies injected with the same volume of nanopure water maintained perfect schooling, 100% survival, and no signs of neurological disturbance. An effective concentration of 4 μg DA/g has been reported in IP studies with 24 g mice, suggesting a similar neurologic sensitivity between mammals and fish. Our results suggest that anchovies are probably effected by DA during toxic events in the same way as higher trophic levels and that DA may play a role in shaping anchovy populations as it does in sea birds and mammals.

ISOLATION AND LC/MS ANALYSIS OF CIGUATOXINS FROM FISH AND MICROALGAE TO ELUCIDATE THE BIODIVERSITY ALONG THE TROPHIC CHAIN OF CIGUATERA

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A total of 59 toxin fractions were isolated from carnivorous fish (moray eels *Gymnothorax javanicus* and red snappers *Lutjanus bohar*), a herbivorous fish (parrotfish *Scarus gibbus*), and the toxin producing dinoflagellate *Gambierdiscus toxicus* (two monoclonal cultures and a wild bloom). FAB-MS and LC/MS experiments revealed for the first time production of eight toxins by two monoclonal cultures (Tubuai and Rangiroa) of *G. toxicus.* Those toxins were shown to be oxidized in carnivorous fish. Difference in toxin profiles were seen not only between fish species but also between the flesh and the viscera, indicating that monitoring of fish flesh for toxicity should be carried out based on the toxin profiles of the flesh. Some of the toxins found in *G. toxicus* have a primary hydroxyl group and thus were shown to be useful in preparing a protein conjugated antigen.

EFFECT OF POLYAMINES ON GROWTH AND TOXICITY OF *CHRYSOCHROMULINA LEADBEATERI* AND *PRYMNESIUM PARVUM* (HAPTOPHYTES)

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The influence of the polyamines (putrescine and cadaverine) were tested on non-axenic cultures of ichthyotoxic algae *Chrysochromulina leadbeateri* (CLTJ1) and *Prymnesium patelliferum.* Replicate cultures of exponentially growing cells were incubated with or without polyamines under light and temperature controlled conditions. Cell abundance, toxicity (haemolytic and *Artemia salina* assays) were analysed, and behaviour (swimming, cannibalism, phagotrophy) was recorded after 24 and 48h. Addition of putrescine and/or cadaverine (1-110 μM) to the culture medium stimulated growth and resulted in increased cell numbers in *C. leadbeateri* and *P. patelliferum* (putrescine: + 30-50 %, cadaverine: 0-30%). High concentrations of both polyamines (1100 μM) had a negative effect on cell numbers in *P. patelliferum* (no increase) and in *C. leadbeateri* (i.e. up to 90-100 % lysed cells relative to the control cultures). Haemolytic substances were extracted both from the cells and the culture medium. Within the range 0-11μM of putrescine addition, *C. leadbeateri* showed very low haemolytic activity (< 1 Seq μg ml-1). At high concentrations of putrescine (110 and 1100 μM), the haemolytic activity increased up to 10 Seq μg ml-1 in the particulate fraction after 48 h. Additions of putrescine, cadaverine or both polyamines gave similar effects on the haemolytic activity in *P. patelliferum.* Furthermore, the toxicity of *P. patelliferum* towards *A. salina* was enhanced four times at 1100 μM polyamines. *Chrysochromulina leadbeateri* was not found toxic to *A. salina*. Addition of polyamines (> 110 μM) resulted in erratic swimming behavior, hyperactivity of the cells for both species, and a significant increase (0 to 10 % of the population) of cannibalism occurred in *P. patelliferum* cultures. Our results support the hypotheses that (i) polyamines may act as growth enhancing compounds, (ii) the haemolytic/ichthyotoxic substances produced by *C. leadbeateri/ P. patelliferum* and the polyamines (added or derived from bacterial decomposition) may produce a haemolytic-polyamine complexes that could be stable in a colloidal phase and possibly "hypertoxic", (iii) polyamines may favour the mixotrophic growth of *C. leadbeateri/P. patelliferum.*

THE CHANGING FACE OF CIGUATERA: AN AUSTRALIAN PERSPECTIVE

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Ciguatera is a pleomorphic syndrome consisting of a range of gastrointestinal, neurological and cardiovascular signs and symptoms that follow the consumption of warm-water marine fish contaminated with the ciguatoxin class of sodium channel activator toxins. The disease causes fatalities in the Indian Ocean but is rarely fatal in the Pacific Ocean and Caribbean Sea. The severity and duration of illness can be reduced with intravenous mannitol. Two structurally distinct families of ciguatoxins, one from the Pacific and one from the Caribbean, have been identified. All these ciguatoxins (CTX) most likely arise from certain strains of the benthic dinoflagellate, *Gambierdiscus toxicus*. Following blooms of *G. toxicus*, these toxins accumulate in fish through marine food chains to levels that affect human health. Factor(s) influencing such bloom formation are unclear. P-CTX-1, the most potent sodium channel toxin known, is the major toxin in ciguateric carnivorous fish in the Pacific, causing human poisoning at levels of 0.1 ppb (10^{-10} mole P-CTX-1/kg) and above. Toxins produced by other benthic dinoflagellates, including okadaic acid and maitotoxin, have no proven role in ciguatera. The mouse assay is presently used to assess levels of ciguatoxin in fish extracts. Other in vivo assays, including the chicken, mongoose, mosquito, brine shrimp and diptera larvae assays, are less widely used. In vitro cell-based assay, which measure the effects of ciguatoxin-induced sodium channel opening or the inhibition of [^{3}H]-brevetoxin binding, are more sensitive than in vivo methods and have the potential to replace the mouse assay. Analytical detection methods, in particular HPLC/tandem ionspray mass spectrometry, are also under development.Antibody-based assays also hold much potential as cost-effective screens for ciguateric fish but presently appear to suffer from a lack of specificity and sensitivity. A major advance in the management of ciguatera will come when a validated screen is commercially available. This presentation will discuss advances in our understanding of ciguatera since "Hank" Banner initiated modern research into the problem in Hawaii in the late 1950s. Particular emphasis will be given to advances in our understanding of ciguatera in the western and central Pacific.

ELECTROCHEMICAL DETECTION OF *PFIESTERIA PISCICIDA* AND *PFIESTERIA*-LIKE SPECIES

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Newly developed electrochemical methods makes it possible to quantitatively detect low concentrations of DNA or RNA from harmful algal species. We will present the basic theory behind electrochemical detection and describe our initial attempts to design a convenient assay for quantitatively detecting *Pfiesteria piscicida* and the *Pfiesteria*- like species *Cryptoperidiniopsis brodyi* in natural water samples. The electrochemical detection method being developed is a modified sandwich hybridization technique. For this assay to work, species-specific gene sequences that can serve as hybridization targets must be identified. Suitable target sequences have been identified from the ribosomal 18S and ITS regions of *P. piscicida* and *C. brodyi.* A short peptide nucleic acid (PNA) probe homologous to the unique target DNA or RNA sequence is then synthesized, labeled with biotin, and attached to a neutravidn-coated electrode. The PNA sequence of the labeled probe then binds the target RNA or DNA from the lysed cells. This forms the first part of the hybridization sandwich or "bridge". At the same time, a second PNA probe labeled with horseradish peroxidase (HRP) also binds to the target DNA or RNA. The binding of the second probe completes the hybridization sandwich. When hydrogen peroxide and an electron transfer mediator are added to the system, a strong catalytic current is produced from the HRP-labeled probes localized by hybrid formation at the electrode surface. The catalytic current is proportional to HRP bound, which in turn is proportional to the amount of target DNA or RNA present. The electrode detection system is highly sensitive, portable, and inexpensive to manufacture.

THE NOVEL SAXITOXIN BINDING PROTEIN, SAXIPHILIN: ITS PHYSIOLOGICAL ROLE AND APPLICATIONS

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Until several years ago, saxitoxin,s only known receptor was the voltage gated sodium channel via which saxitoxin (STX) mediates its toxicity. STX is produced by marine and freshwater microalgae and can infest commercially harvested and farmed shellfish. These toxic shellfish cause many deaths around the globe each year. Unlike the sodium channel which is membrane bound, multisubunit protein, saxiphilin is a soluble, single subunit protein found in the circulatory fluid of many vertebrates and invertebrates. Saxiphilin is in fact a transferrin, proteins that bind, transport and deliver cellular iron in many animals. Some animals found to date to possess saxiphilin include lizards, amphibians, fish, spiders, insects and centipedes, many of which are not known to harbour or accumulate STX. No bird or mammal has yet to be found to possess saxiphilin. Interestingly, not all members of a particular taxonomic group will possess saxiphilin and it is unpredictable as to what species will be found to be a saxiphilin producer. STX has only ever been found in the marine and freshwater environment. Why then do so many terrestrial organisms possess saxiphilin? For example, why does a tropical centipede found in the leaf litter of forests, need such an STX binding protein? Does STX occur more widely in the environment than presently thought selecting for the evolution of saxiphilin? Is saxiphilin a form of chemical defence against a noxious compound encountered by the organism in its day to day life? Or does saxiphilin play a more complex physiological role modulating a hormone like molecule? To uncover what its physiological role may be, different isoforms of saxiphilin have been pursued and characterised. As a byproduct of this effort, isoforms have been disocvered with attributes which make them amenable to practical applications. A case in point is the isoform from a tropical centipede that has an affinity for STX so strong that the half time for dissociation of STX from the protein is almost 2 days. This protein has been used to develop a rapid-throughput, microtitre plate assay using radiolabelled STX which can be used to specifically detect STX and its many natural variants. This assay is robust with regards potential interference by contaminating compounds encountered in biological extracts.

HARMFUL ALGAL BLOOM RESEARCH AND MONITORING IN NEW ZEALAND: AN OVERVIEW OF THE 1990S

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Since 1993 there have been substantial research and monitoring efforts in New Zealand focussed on determining the nature, incidence, causes and effects of harmful algal blooms (HABs) in the marine environment. During that time approximately $NZ 20 million has been spent on nation- wide government and shellfish industry sponsored monitoring programmes and about $NZ 5.5 million on research. These efforts have provided a good perspective on the problem and permitted informed judgements on a variety of important questions. Do HABs present an important public health risk? Have HAB phenomena become more prevalent with increased developmental pressure, including aquaculture, on the coastal environment? Is there evidence of the introduction of exotic HAB species? Can we predict toxic bloom events? What are the prospects for control and mitigation? Do HABs pose a significant problem for the further development of the aquaculture industry? What are the future directions for research ? Where do we hope to be in 2010 in terms of new techniques and systems for monitoring HABs and marine bio-toxins? These and other questions of relevance to the HAB research community, government regulators and the shellfish industry will be discussed.

COMPARATIVE EFFECT OF HIGH LIGHT INTENSITY ON *CHATTONELLA MARINA* FROM SOUTH AUSTRALIA AND JAPAN : IMPLICATIONS FOR REACTIVE OXYGEN SPECIES PRODUCTION.

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The raphidophyte *Chattonella marina,* is a known ichthyotoxic species associated with fin-fish mortalities in aquaculture. The loss of $A45M of farmed bluefin tuna in South Australia in 1996 raised the question of differences in toxicity between the Australian and Japanese strains of *C. marina*. Autecological studies demonstrated that the Japanese and Australian strains had similar tolerances for temperature and salinity, but substantial differences in irradiance requirements, with the Australian strain showing accelerated growth under irradiances of 400 μmol m-2 s-1 (Marshall & Hallegraeff. 1999. J. Plank. Res. 21, 1809-1822). This adaptation to high light intensity related to the presence of the Mycosporine- like amino acids (MAAs), shinorine, mycosporine-glycine, and mycosporine-glycine:valine, at levels higher in the Australian strain than the Japanese strain, with mycosporine-glycine absent in the Japanese strain. Mycosporine-glycine, a noted antioxidant, may reduce the effect of the reactive oxygen species (ROS) in the Australian strain. The effect of irradiance on ROS production was determined for both the Australian and Japanese strains using the zooplankton *Artemia salina* and fish *Onchorynchus mykiss* as bioassays. The relative importance of ROS production and neurotoxins as fish killing mechanisms by this alga are discussed.

DOES SALMONOID AQUACULTURE IMPACT BLOOMS OF *ALEXANDRIUM, PSEUDO-NITZSCHIA* AND *DINOPHYSIS?*

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Salmon farming began in the southwest Bay of Fundy, eastern Canada in 1978 and has since expanded to include approximately 85 operations with an annual market value exceeding $130 million (Can). There is increasing concern that intensive aquaculture is being linked to increased eutrophication and industrial pollution as well as concern that resulting conditions may enhance growth of harmful algal blooms. Phytoplankton populations as well as various chemical and physical parameters have been studied throughout the year at 4 sites in the southwest Bay of Fundy since 1988. In addition data for paralytic shellfish toxins in shellfish dates back to 1944 and domoic acid levels have been documented since 1988.

Silicate values ranged from 0.27 μM in the summer to 14.63 μM in the winter; phosphate levels varied between 0.073 and 1.571 μM; nitrate values of 0.02 μM in September to a high of 10.47 μM in winter were measured and ammonia varied between 0.60 and 10.02 μM. Phosphate and ammonia levels tended to be slightly elevated in aquaculture areas. Blooms of *Alexandrium fundyense* are seeded from the offshore waters (where greatest cell densities have been observed) and occur during June and July. Highest concentrations were observed during 1989 and '90 with more than 70,000 cells/L and during the past 4 yr, concentrations have not exceeded 3,000 cells/L. Shellfish toxicity data indicates that highest PSP toxicities were detected during 1944. Domoic acid reached levels above the regulatory limit in shellfish during 1988 and 1995 when concentrations of *Pseudo-nitzschia pseudodelicatissima* exceeded 1 million cells/L. The 1988 bloom was initiated in situ in a location where there were no aquaculture sites and the 1995 bloom was advected to the inshore aquaculture region.

Results indicate that populations of *A. fundyense, P. pseudodelicatissima* and *Dinophysis* spp. in the Bay of Fundy region are not influenced by the aquaculture industry nor have they grown in intensity in recent years or since the advent of aquaculture. However, other species, not presently known to cause harm appear to be increasing throughout the region.

HARMFUL DINOFLAGELLATE CYSTS FOUND IN SURFACE SEDIMENTS AND A CORE SAMPLE COLLECTED FROM CHANJEAN RIVER, CHINA

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An influence of human activities to coastal marine ecosystem has become greater recently. Blooms of harmful and toxic plankton called as red tides and shellfish poisonings are closely related with increase of nutrients such as phosphorus and nitrogen flowing into coastal waters from land, and are typical phenomena effected by human activities such as industrialization an urbanization. Dinoflagellates is one of important causative organisms for red tides and shellfish poisonings, and so many studies have been carried out on modern dinoflagellates from this viewpoint. The Chanjean River mouth is one of the famous coastal areas where the amount of an artificial load increases remarkably by great economic activities derived from extremely high dense population in recent years. As a result, harmful and toxic plankton blooms frequently occur in coastal waters of these areas. In this paper, palynomorph assemblages including harmful dinoflagellate cysts in surface sediments obtained off Chanjean River mouth are first clarified to know the present situation of water environment. And then we will understand an temporal environmental change, especially water quality and harmful and toxic dinoflagellate blooms based on the change of dinoflagellate cyst assemblages in a core sample which record more than last three decades.

ALGICIDAL BACTERIA ACTIVE AGAINST *GYMNODINIUM BREVE*

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A growing body of evidence suggests that algicidal bacteria may play an important role in naturally regulating the development and termination of harmful algal blooms (HABs). Interest in such microbes has been enhanced further by their potential use as part of a HAB management strategy. We have isolated two bacterial strains from the west Florida shelf that are lethal to *Gymnodinium breve*, a bloom-forming dinoflagellate responsible for severe economic losses in this region through impacts on fisheries and tourism. A previous report on these bacteria described aspects of their killing activity and the taxonomic specificity of target organisms. We have now completed sequencing of the 16S rRNA gene for both algicidal bacteria and preliminary analyses suggest that one strain resides in the flexibacter-cytophaga subgroup of the cytophaga/flexibacter/bacteroides (CFB) phylum, while the other strain is a member of the gamma-proteobacteria. Fluorescently-labeled rRNA probes have been developed for both taxa and are being employed via in situ hybridizations on both laboratory and field samples to begin characterizing the population dynamics and distribution of these algicidal strains. Progress is also being made toward the identification of the algicidal compound(s) produced by one of the bacteria. A high-throughput bioassay for guiding fractionation of extracellular bacterial metabolites based on algicidal activity has been developed. Using this approach, chromatographic separation of an algicidal component from bacterial culture filtrates has been achieved, and further purification as well as structural analyses using mass spectrometry and NMR are underway. We will present a summary of laboratory and field results to date describing these *G. breve* killing bacteria and their algicides. Finally, a consideration of all algicidal bacteria reported thus far from different geographical regions will provide evidence for the ubiquitous nature and close phylogenetic affinities of these microbes, and thus their potential importance as regulators of HAB dynamics.

DOMINANCE OF *CYLINDROSPERMOPSIS RACIBORSKII* IN QUEENSLAND TROPICAL AND SUBTROPICAL RESERVOIRS: IMPLICATIONS FOR MONITORING AND MANAGEMENT

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Since October 1997, 47 reservoirs and weir pools across tropical and sub-tropical Queensland have been regularly monitored for the occurrence of cyanobacteria. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju was found in 70% of the storages, with one storage displaying year-round dominance, 50% of the reservoirs seasonally dominated, and a seasonal presence in 46% of the weir pools. Maxima for the majority of storages occurred from late summer through to early autumn. The precise timing of onset of seasonal maxima varied considerably between storages and regions. Temperature and stratification patterns influenced seasonal recruitment with *C. raciborskii* reaching seasonal maxima in southern storages generally later than the northern storages. Overall peak seasonal abundance occurred in deep strongly stratified storages.

The majority of storages experiencing concentrations > 15 000 cells mL-1 tested positive to the presence of the alkaloid cytotoxin,cylindrospermopsin. Median cylindrospermopsin concentration across the fourteen reservoirs in which toxin was recorded was 3.4 μg L-1. The highest toxin concentrations were generally associated with storages in which *C.raciborskii* had been established for a considerable period of time, or occurred after the peak summer population maxima. Toxin concentrations of 1 μg L-1 were generally associated with cell concentrations of ca. 20 000 cells mL-1, hence this cell concentration threshold was adopted as a health trigger level at which to begin monitoring for toxicity.

The morphology of this species was highly variable and included straight, coiled, and sigmoid-shaped trichomes. Populations were routinely recorded as mixtures of all three morphotypes or proceeded as transitions from one morphotype to another throughout the year. The dominance of *C. raciborskii* appears to be favored by a set of environmental and hydrological factors including long water residence time, high pH, high temperature, high incident irradiation and a thermally stratified water column.

The lack of visual monitoring cues such as scum formation, variation in colour of the water body, rapid germination of large numbers of cells, highly variable morphology, relative toxicity and persistence of this species year round in many areas continues to make this species a primary focus of water managers in this state.

RECENT APPEARANCE OF *GYMNODINIUM CATENATUM* AT PORT LINCOLN, SOUTH AUSTRALIA?

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In association with a major tuna kill in Boston Bay, Port Lincoln, SA, in 1996 , an intensive phytoplankton survey identified the presence of the toxic dinoflagellate *Gymnodinium catenatum* at this site for the first time. *Gymnodinium catenatum* had not previously been recognised in SA and has only been identified from a few sites on the mainland of Australia (notably Victorian coastal waters). Genetically and toxicologically the Port Lincoln and Tasmanian dinoflagellate populations are indistinguishabele, and this project therefore seeks to determine whether the Port Lincoln site is the origin of the recently introduced Tasmanian population (since 1971) or whether the population at Port Lincoln has been secondarily introduced from Tasmania (i.e. via a known domestic shipping link).Three sediment cores were taken from Boston Bay. Each contained abundant *Gymnodinium catenatum* cysts in the top 6 cm but these disappeared rapidly with depth. ^{210}Pb profiles indicate that bioturbation extends down to no more than 5 cm in each core and that their sedimentation rate is approximately 0.2 cm/yr. Modelling of the cyst and ^{210}Pb profiles suggests that the observed profiles are consistent with an introdutction event within the last 25 years. This approach confirms that *Gymnodinium catenatum* was newly introduced to Port Lincoln but does not provide the temporal resolution to determine whether or not this could be the source of the Tasmanian populations.

TOXIC *ALEXANDRIUM TAMARENSE* ISOLATES (DINOPHYCEAE) FROM THE ORKNEY ISLANDS, SCOTLAND, ARE RELATED TO NORTH AMERICAN STOCKS

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Isolates of the *Alexandrium tamarense* (Lebour) Balech species-complex taken during a bloom at the Orkney Islands, north of Scotland, in May 1997 have been examined morphologically and can be assigned to the morpho-species of *A. tamarense* rather than A. fundyense Balech. All isolates have been tested for toxicity using HPLC analysis and found to be as toxic as the most toxic *A. fundyense* isolates from North America. A subset of these isolates has been genetically analyzed using an AFLP DNA fingerprinting assay. We used this analysis to evaluate the hypothesis by Scholin et al. (1995) that dispersal of ancestral populations from the Pacific entered into the North Atlantic with the opening of the passage between Canada and Greenland or the hypothesis put forward by Medlin et al. 1997 that the populations entered the Atlantic with the opening between Greenland and Svalbard. Probes for the entire *Alexandrium tamarense* species-complex based on 18S rRNA sequence data were constructed and compared with those for the toxic `North American clade and the non-toxic Western European clade. Their use on field material from the 1997 bloom was also evaluated with flow cytometric detection. This work was supported by the German BMBF TEPS 03F0161 project.

ON THE LONG-TERM RESPONSE OF HARMFUL ALGAL BLOOMS TO THE EVOLUTION OF EUTROPHICATION OFF THE BULGARIAN BLACK SEA COAST: ARE THE RECENT CHANGES A SIGN OF RECOVERY OF THE ECOSYSTEM? — THE UNCERTAINTIES

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Based on the level of eutrophication of the Black sea coastal ecosystem the 1954 – 1998 period could be subdivided into: a relatively pristine period (60-70ies), a period of an intensive anthropogenic pressure (the 80-ies), and the 90-ies a period of relaxation, related to the collapsing economy and agricultural production and the concurrent reduction of the land-based nutrients load to the basin. In the present paper the long-term trends (1954-1998) of phytoplankton blooms (species involved, frequency and timing) are discussed in relation to the evolution of anthropogenic eutrophication and the variability of temperature, sun activity and total zooplankton biomass. The main peculiarities of the current period – an increase in the diversity of red-tide species; a shift in their taxonomic composition, a decrease of blooms frequency and maximum densities attained; a reduction of summer blooms on the account of an increase in winter-spring and autumn events, coincide well with the decrease of nutrients level and the shift of their ratios especially in summer. The MDS plot of phytoplankton blooms and the environmental matrix reveal similar clustering discriminating between 70ies-90ies and 80ies, the 90-ies being more close to the 70-ies. The results suggest that the recent changes of phytoplankton blooms could be considered a sign of recovery as a response to the relative improvement of the chemical parameters of the coastal zone. The main uncertainties are still the maintained capacity of the ecosystem to produce high biomass, the concerted environmental changes and similar alterations reported for other regions of the World Ocean, suggesting a possible global climatic signal of influence too.

FORMATION OF CHLOROPHENOLS DURING TREATMENT OF WATER CONTAMINATED WITH CYANOBACTERIA.

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Occurrence of toxic cyanobacteria in drinking water supplies has become an important problem for the water authorities. Recent research demonstrated that cyanobacterial toxins such as microcystin LR, cylindrospermopsin and saxitoxin are successfully degraded to below the detection limit if the water is treated with chlorine, providing a residual free chlorine concentration greater 0.5 mg L-1 is maintained for sufficient time. The aim of this study was to determine formation of chlorinated byproducts during the treatment of water spiked with environmentally relevant concentrations of cylindrospermopsin and microcystin. Experiments were set-up in which water (20 – 60 L) was spiked with cell free extract material of toxic *Microcystis* and *Cylindrospermopsis*. The water samples, including control samples (nonchlorinated water with no algae, chlorinated water with no algae and nonchlorinated water with algae) were then filtered and/or passed through a XAD-2 solid phase cartridge under vacuum. For chlorophenols the water was acidified (pH < 2) prior to sampling. Samples were then subject to GC-MS analysis. Results showed that formation of lower chlorinated phenols (mono – trichlorophenol) during the treatment of the water which contained the cell free extract material has occurred.

CYANOBACTERIAL PHYLOGENY AND THE EVOLUTION OF CYANOTOXICITY

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Non-ribosomal peptide synthesis is achieved, in prokaryotes and lower eukaryotes, by the thiotemplate function of large, modular enzyme complexes, known collectively as peptide synthetases. These and other multifunctional enzyme complexes, such as polyketide synthases, are of interest due to their use in unnatural product or combinatorial biosynthesis (McDaniel, R., S. Ebert-Khosla, D. A. Hopwood, and C. Khosla. 1993. Science 262:1546-1557; Stachelhaus, T., A. Schneider, and M. A. Marahiel. 1995. Science 269;69-72). Most non-ribosomal peptides from microorganisms are classified as secondary metabolites, that is, they rarely have a role in primary metabolism, growth, or reproduction but have evolved to somehow benefit the producing organism. Cyanobacteria produce a myriad array of secondary metabolites, including alkaloids, polyketides, and non-ribosomal peptides, some of which are potent toxins. This paper addresses the molecular genetic basis of non-ribosomal peptide synthesis in diverse species of cyanobacteria. Amplification of peptide synthetase and polyketide synthase genes is achieved using degenerate primers directed to conserved functional motifs of these modular enzyme complexes. Specific detection of the gene cluster encoding the biosynthetic pathway of the cyanobacterial toxin microcystin was shown in both cultured and uncultured samples. DNA amplifications, sequencing, and evolutionary analysis revealed a broad distribution of peptide synthetase gene orthologues in cyanobacteria. The results demonstrate a molecular approach to assessing pre-expression microbial functional diversity in uncultured cyanobacteria. Only one cyanobacterial genus studied to date shows a relationship between taxonomic affiliation and the genetic basis behind toxicity. Extensive lateral transfer of the genomic loci responsible for toxin biosynthesis is proposed. The non-ribosomal peptide biosynthetic pathways detected may lead to the discovery and engineering of novel antibiotics, immunosuppressants, or antivirals.

POPULATION DYNAMICS OF THE TOXIC DINOFLAGELLATES *DINOPHYSIS* SPP. IN MAIZURA BAY, JAPAN, WITH SPECIAL REFERENCE TO AUTOFLUORESCENCE CHARACTERISTICS AND ATTACHMENT OF PICOPHYTOPLANKTON

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Population dynamics of the dinoflagellates *Dinophysis* spp., were investigated in Maizuru Bay, Japan, from May 1997 to August 1999. Seven species of *Dinophysis* were detected including toxic speices of *D. acuminata* and *D. fortii.* The most dominant species of *Dinophysis* was *D. acuminata*, which was detected throughout the year and abundant during the period of water temperatures between 15 and 18 degrees. Phycoerythrin containing nano and picophytoplankton (cryptophytes and cyanobacteria) were enumerated simultaneously. No relationships were found among the cell densities of *Dinophysis* spp. and these small plankton. Autofluorescence characteristics and attachments of picophytoplankton cells to *Dinophysis* cells were also observed. Autofluorescence of *Dinophysis* spp. (mainly *D. acuminata* and *D. fortii*) under blue-light exitation was yellow-orange in general. Occasionally, *Dinophysis* spp. had particles with red autofluorescence together with yellow-orange autofluorescence. Cells only with red autofluorescence were also observed in *D. acuminata* and *D. fortii. Dinophysis* cells containing red autofluorescence were relatively abundant from spring to early summer. We noticed that many coccoid cells of picophytoplankton (ca. 1-2μm in diameter) were attached to the cell surface of *D. acuminata, D. fortii*, etc., and food vacuole-like structures were also sometimes observed. These observations suggest a close relationship between mixotrophic *Dinophysis* spp. and picophytoplankton.

PARVILUCIFERA INFECTANS (NOREN AND MOESTRUP 1999): A PARASITIC FLAGELLATE CAPABLE OF KILLING TOXIC MICROALGAE

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A natural population of the toxic dinoflagellate *Dinophysis*, was found to contain an unknown parasitic protozoan, named *Parvilucifera infectans* (Norén et Moestrup 1999). The identity of the parasite was examined by a combination of light- and electron microscopy and DNA sequencing. It was found to be related to *Perkinsus*, an oyster killing protist. Infection studies showed that the *Parvilucifera infectans* also infects and kills several other dinoflagellates, notably *Alexandrium.* The discovery of an organism that is a lethal parasite to two of the worlds most harmful genera of dinoflagellates is an important finding. This could be a breakthrough for our possibilites to control harmful algal blooms (HAB:s). Researchers around the world have been looking for options to control HAB:s during the last decades which includes various methods but so far little progress has been made and pleas for more concerted efforts to search for control organisms of HAB:s have been made. *Parvilucifera infectans* has the two prerequisites needed on industrial scale to control HAB:s or otherwise toxic algae; (1) they have high efficiency of the lethal infection and (2) the possibility to be cultured on a larger scale. A successful HAB treatment with *Parvilucifera* would shift the plankton community towards non-toxic athecate dinoflagellates or other plankton groups. Valuable nutrients are not removed from the water column (as a source for the primary producers) but are channeled into non-toxic algae. This, of course, is hypothetical and intense research is needed to judge if this is a possible way of controlling HAB:s. But compared with methods such as the use of chemical pesticids, a natural occurring control organism seems to be preferable for the environment.

HARMFUL PHYTOPLANKTON EVENTS CAUSED BY VARIABILITY IN THE IRISH COASTAL CURRENT ALONG THE WEST OF IRELAND.

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Frequent sampling in summer along the western and northwestern coasts of Ireland showed the rapid onshore development of blooms of potentially harmful phytoplankton species. In both 1998 and 1999, concentrations of *Gyrodinium* cf. *aureolum* rose by four orders of magnitude to over one million cells per litre in Donegal Bay (northwestern Ireland) in less than 10 days. The rapid development of these populations was linked to advection resulting from unfavourable wind-forcing of the Irish Coastal Current (ICC) which runs northwards along the western Irish coast. Current measurements showed that after a particular sequence of changes in wind direction phytoplankton populations could be rapidly advected from areas of slack circulation on the shelf via the ICC into aquaculturally sensitive coastal zones such as Donegal Bay. The model presented is similar to one already demonstrated for the occurrence of toxic events in the bays of southwestern Ireland. Other historical harmful events along the west and northwest coasts relating to substantial losses in both finfish and shellfish culture could also be explained using the model. These include the *G. aureolum* bloom of 1992, the *Prorocentrum balticum* bloom in 1997 as well as discoloured water caused by a bloom of *Noctiluca scintillans* in 1999.

DEVELOPMENT OF MOLECULAR DETECTION METHODS FOR *PFIESTERIA PISCICIDA* AND OTHER ESTUARINE TOXIN-PRODUCING DINOFLAGELLATES GUIDED BY HETERODUPLEX MOBILITY ASSAY ASSISTED SEQUENCE DISCOVERY.

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Fish kills and possible adverse human health effects have been attributed to *Pfiesteria piscicida* and other toxin producing estuarine heterotrophic dinoflagellates in US mid-Atlantic estuarine waters. Identification of these organisms in field samples (and, for that matter, in dinoflagellate culture) is problematic, and currently requires screening by light microscopy for the presence of *'pfiesteria*-like-organisms'; cultivation in the presence of fish as prey species (to confirm toxicity); and culture amplification to permit scanning electron microscopy (for definitive morphological identification based on thecal plate configuration). Genetic characterization of these organisms has been hampered by the lack of an axenic culture system. We adopted a molecular ecological approach utilizing PCR primers with Dinophyte specificity targeted to 18s rRNA gene sequences. These primers, whose specificity was demonstrated against an array of eukaryotic algal and protozoal species, were used for specific amplification of dinoflagellate gene sequences among complex genomic pools (non-axenic algal/dinoflagellate cultures and field samples of estuarine waters). Utilizing DNA extracted from active fish-kill bioassay cultures and environmental samples collected during fish kill events, dinoflagellate sequence diversity within amplified dinoflagellate 18s gene sequences was assessed by heteroduplex mobility assay (HMA), single stranded conformational polymorphism (SSCP), and sequencing. Use of the HMA was particularly helpful in identifying mixed dinoflagellate cultures amongst panels previously believed to be unialgal. A particular HMA pattern was consistently identified among cultures (nine) identified both by toxicity and scanning electron microscopy as *Pfiesteria piscicida.* PCR products with this signature pattern were cloned and sequenced, leading next to identification of the full-length 18s DNA sequence of *Pfiesteria piscicida. P. piscicida* specific PCR primers were then developed, and their specificity confirmed against panels of known dinoflagellate species. A real-time PCR assay (TaqmanR) has been developed to permit rapid and quantitative identification of the organism in estuarine waters.

BLOOMS OF THE TOXIC CYANOBACTERIA *LYNGBYA MAJUSCULA* IN COASTAL QUEENSLAND WATERS

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Over the past few years (1996-present) large blooms of the cyanobacterium *Lyngbya majuscula* (Gomont) ("Mermaid hair") have been identified during the summer months in several coastal environments in southeast Queensland, Australia. *Lyngbya majuscula* is a non-heterocystous, nitrogen-fixing cyanobacterium that forms of 10-30 cm long filaments which grow loosely attached to seagrasses and macroalgae, and has been shown in other regions to contain a suite of toxins. The largest *L. majuscula* bloom, in a northern embayment of Moreton Bay (Deception Bay), covered an area of approximately 7 km^2, mostly in water depths < 3m with sandy sediments.

Human health impacts that have been associated with these *Lyngbya majuscula* blooms include severe contact dermatitis causing affected skin to blister and peel off, eye irritation and asthma-like respiratory distress. The asthma-like respiratory symptoms have been reported by fisherman working with crab pots or ropes with dried *L. majuscula*, which forms a fine, easily aerosolised powder. There have been additional reports of similar symptoms from fisherman in contact with blooms off the coast of Bundaberg and Harvey Bay, as well as from swimmers and tourists off the oceanic beaches of Fraser Island. Extracts from *L. majuscula* in Moreton Bay have elicited rapid and strong responses using mouse ear swelling tests. Histological examination of mice injected with extracts of the cyanobacteria showed slight vacuolation at the surface of their kidneys.

Observed ecological health impacts of *Lyngbya majuscula* blooms in SE Queensland include: i) localised seagrass loss, ii) poor crab and fish harvests (reported by fishermen) compared with non-*Lyngbya* years, iii) localised input of bioavailable nitrogen through *L. majuscula* nitrogen fixation, iv) large beach wracks of decaying *L. majuscula* emitting a putrid odour necessitating the removal of the decomposing material by local authorities in the Deception Bay region. We hypothesise that *Lyngbya* blooms may be related to localised alterations of the micronutrient iron (Fe), as well as interaction with phosphorus (P).

INGESTION OF TOXIC *MICROCYSTIS AERUGINOSA* BY DAIRY CATTLE AND THE IMPLICATIONS FOR MICROCYSTIN CONTAMINATION OF MILK

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Microcystin (MCYST) toxins produced by the water bloom forming cyanobacterium *Microcystis aeruginosa* are chemically stable compounds which have both acute and chronic health effects on mammals, including cattle and humans. Cattle will drink water containing lethal cell concentrations of *M. aeruginosa.* When cattle consume sub-lethal doses of microcystins, the ultimate fate of those toxins is unknown. We undertook a study to examine the transmission of microcystins from drinking water containing environmentally realistic concentrations of *M. aeruginosa* (strain MASH01-A19) through lactating dairy cattle to determine if the MCYST-LR toxins present in the cyanobacteria, could subsequently be detected in milk produced by those cattle. During the course of the study, cattle consumed up to 15mg of highly hepatotoxic MCYST-LR over a three week period. In this talk we will release the outcomes of our findings and the implications for contamination of milk products by microcystins resulting from ingested cyanobacteria.

SEDIMENTOLOGICAL EVIDENCE OF AN INCREASE IN *PSEUDO-NITZSCHIA* (BACILLARIOPHYCEAE) ABUNDANCE IN RESPONSE TO COASTAL EUTROPHICATION

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Pseudo-nitzschia abundance is extremely high in the northern Gulf of Mexico in the plume of the Mississippi River, especially when river flow and nutrient inputs are high. Five sediment cores were collected to determine if, and to what extent, *Pseudo-nitzschia* valves preserved in the sediment in order to reconstruct changes in *Pseudo-nitzschia* abundance in the past century. *Pseudo-nitzschia* increased in relative and absolute abundance in all five sediment cores, most markedly in the sediment layers corresponding to the time period between the 1950s and 1970s as determined by lead-^{210} sedimentation rate estimates. Previous research has demonstrated that a dramatic increase of fertilizer use in the United States occurred at this time, which has resulted in the development of eutrophication and hypoxia in the coastal waters of the northern Gulf of Mexico. Scanning electron microscope analysis of the fine structure of *Pseudo-nitzschia* and other lightly-silicified diatom valves preserved in the sediment indicates that the increase in *Pseudo-nitzschia* abundance appears to reflect a response to eutrophication rather than valve dissolution processes. This study provides evidence for a direct link between coastal eutrophication and harmful algal blooms.

AN AGENDA TO MINIMISE THE SPREAD OF HARMFUL ALGAL BLOOMS BY SHIPPING

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The role of shipping in the transport of organisms that can result in harmful algal blooms and hence the increasing threat posed by such organisms has been on the agenda of the International Maritime Organisation (IMO) since 1973. It was in this year that the World Health Organisation referred the matter to IMO due to "possible implications for human health". The IMO's Marine Environment Protection Committee (MEPC) commenced detailed consideration of the matter in 1990 and has since that time, taken many significant steps. The focus in IMO in this matter has been largely related to ballast water. Most ships must regularly take on and discharge ballast water. When not carrying cargo, ships need ballast water to enable them to operate effectively and safely. Some bulk carriers that visit Australia to take on cargo bring with them vast amounts of ballast water- over 100,000 tonnes-from foreign ports then on arrival in Australia, or in another foreign port, discharge this ballast water and take on cargo. Globally, it has been estimated that the world shipping fleet is transporting approximately 10 billion tonnes of ballast water arounf the globe each year and that, on average, more than 3,000 species of plants and animals are being transported daily around the world (UNDP report 1998).

The organisms which result in toxic algal blooms are quite readily able to be taken up in ships' ballast water, survive during voyages and stand some chance of survival and establishment in their new location when discharged with ballast water. In turn, they will, under suitable conditions, reproduce and potentially become an algal bloom. Following on from international guidelines issued by IMO in 1993, MEPC has in recent years been developing an international regulatory regime which would result in improved ballast water management and thus the reduction of the threat posed by the introduction and establishment of these organisms. Additionally, many countries including Australia have moved or are moving unilaterally to put their own arrangements in place. Now there is no question that shipping is essential to world trade, indeed it moves some 80% of world commodities; in Australia's case it is some 98%. Equally, there is no question that ships need ballast water. The question then is what management arrangements can be put into place to minimise the risk of uptake, discharge and potential spread of unwanted harmful aquatic organisms and pathogens. This address will provide some background to the relationship between the shipping industry and harmful algal blooms and details of key international action taken and contemplated by IMO.

THE REVERSED MICELLAR MEDIUM AS A UNIVERSAL TOOL FOR THE DEVELOPMENT OF ANTIBODY-BASED ASSAYS TO MARINE PHYCOTOXINS USING SMALL AMOUNT OF MATERIAL

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Marine phycotoxins encompass a large class of usually low molecular weight and chemically different compounds ranging from polyethers to alkaloids and peptides or even amino acids. These potent toxins are produced by a number of microorganisms and accumulate in seafood. Due to public health requirements and seafood industries interests, toxin detection has been developed using the mouse bioassay and more specific assays based upon either instrumental methods (gas or liquid chromatography coupled or not to mass spectrometry and capillary electrophoresis), or pharmacological and immunochemical techniques. Among these detection methods, enzyme-immunoessay (EIA) offers the advantages of a technique applicable to all toxin classes not requiring sophisticated and expensive facilities for implementation into routine monitoring program of seafood matrices. Nevertheless, production of specific antibodies is hampered by considerations such as toxicity, scarcity or chemical nature of toxins. This work describes the successful preparation of hapten-protein immunogenic conjugates in a reversed micellar medium using small amounts (0.32-0.64 μmol) of haptens bearing hydroxyl or carboxyl groups. This procedure has been first validated using molecular models and then applied to purified toxins such as brevetoxin (PbTx-3) and domoic acid or a synthetic ring fragment of ciguatoxin (CTX). Epitope density of conjugates ranging from 15 to 25 have been determined using a combination of chromatographic, spectrphotometric , chemical , biological and radiochemical techniques, depending upon the hapten used. Following mice immunisation with the corresponding conjugates, highly hapten specific antibodies have been produced with KD values in the range of 10^{-6} -10^{-8} M. These results confirmed the potential in preparing immunogen with very rare haptens whose low detection level still remains problematic. As the principal requirements for toxin immunodetection in food matrices are sensitivity and specificity, efforts must also be made to set rapid and efficient extraction procedures.

FLOW CYTOMETRIC APPLICATION OF MONOCLONAL ANTIBODY AND RRNA PROBES ON FLOW CYTOMETRIC APPLICATION OF MONOCLONAL ANTIBODY AND RRNA PROBES ON CULTURED *GYMNODINIUM MIKIMOTOI* (DINOPHYCEAE) AND *PSEUDONITZSCHIA MULTISERIES* (BACILLARIOPHYCEAE) IN RELATION TO NUTRIENT STATUS

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Batch cultures of *Gymnodinium mikimotoi* and *Pseudonitzschia multiseries* were sampled in nutrient-replete and nutrient-stressed conditions to examine the variability of label intensities of two different types of probes: (i) species-specific monoclonal antibodies (MAb) against cell surface antigens, and (ii) universal and *P. multiseries*-specific rRNA-targeted DNA probes. All probes were conjugated to FITC and label intensity was measured on a per cell basis as FITC-fluorescence (FBG) using flow cytometry. For *G. mikimotoi* the mean FBG intensity of the MAb-probe was 3 times that of the RNA-probe. Both the MAb and the RNA probe showed no significant decrease in label intensity under nitrogen stress, although a small (<10%) but significant decline in the FBG(RNA):FBG(MAb) ratio could be detected. The applicability of this ratio in the measurement of species-specific in situ nitrogen limitation is discussed. *P. multiseries* could only be labelled with the rRNA-probes. FBG intensities of cells labelled with these probes declined significantly in relation to nutrient stress. Only nutrient-replete *P. multiseries* cells had FBG-values high enough for flow cytometric discrimination against non-labelled controls, which has implications for the applicability of this method in field samples. For both monoclonal and RNA-label methods, information is provided on (i) sample stability in relation to fixation and (ii) label stability of FITC-probed cells. Cell recoveries were independent of the fixation method (formaldehyde or saline ethanol) or nutrient status of the cells. However, recoveries ranged from only 50-60% (*G. mikimotoi)* to 30-40% *(P. multiseries*) which emphasises the need for improving the methods used.

UPTAKE, EFFECTS AND METABOLISM OF THE CYANOBACTERIAL TOXIN MICROCYSTIN-LR ON THE EMERGENT REED PLANT *PHRAGMITIS AUSTRALIS* (CAV.) TRIN. EX STEUD

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Among the most productive ecosystems are macrophyte-dominated lakes. Emergent and submerged macrophytes have an outstanding ability to assimilate nutrients and also create optimal conditions for the microbial decomposition of organic matter. Massive growth of cyanobacteria (blue-green algae) can also develop in such eutrophic waterbodies. From many of these cyanobacteria, it is well known that they can produce a wide range of toxins of which the microcystins are the most common. The common reed, *Phragmitis australis* is abundant and often dominates the flora in freshwater wetlands, but in recent years a decrease in *P. australis* has occurred. It has been shown that cyanobacterial blooms have negative effects on shoot length and dry weight of *P. australis*. In this study, the possible uptake of microcystin-LR (MC-LR) in different cormus parts of *P. australis* was investigated showing that the lower part of the stem was the region with the highest uptake in comparison to the rhizom/root system. Furthermore, transportation of the toxin in this plant species could be shown. The activity of the microsomal and soluble glutathione S-transferases as the first step reaction in detoxication of MC-LR, was investigated and MC-LR-glutathione conjugates were detected by HPLC. The activity of these enzyme systems varies in the different cormus parts with highest activity for the conjugation of MC-LR in the stem.

GYMNODINIUM BREVE TOXINS WITHOUT CELLS: EXTRA-CELLULAR AND INTRA-CELLULAR TOXINS.

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The dinoflagellate, *Gymnodinium breve*, produces several neurotoxins that cause neurotoxic shellfish poisoning, massive fish kills and respiratory irritation in marine mammals and humans. The common method for discerning dangerous concentrations of *G. breve* toxins for public health advisories is enumeration of live cells in the water. Evidence for viability of the neurotoxins outside of cells indicates that shellfish contamination and finfish intoxication could result from water masses carrying suspended/dissolved neurotoxins in the absence of viable *G. breve* cells. Therefore, reliance on cell counts only for public health protection and for discerning toxin fate and effects in the marine environment may be insufficient under some scenarios requiring toxin analyses in addition to cell counts. Studies were performed using laboratory cultures of *G. breve* as well as during natural *G. breve* bloom events in the Gulf of Mexico. These studies provided quantitative and qualitative analysis of extra-cellular toxins (outside *G. breve* cells) and intra-cellular toxins (inside the cells) showing changes in toxin composition over time and space for the natural blooms and through cell growth periods in the laboratory cultures. An important aspect to note is that the extra-cellular toxins are the form available for bubble-mediated transport to the sea surface with subsequent incorporation into marine aerosol. Thus the extra-cellular toxin concentration may be the important component in assessing the potential for adverse respiratory impacts on marine mammals and public health.

PARALYTIC SHELLFISH POISONING IN THE ABALONE *HALIOTIS MIDAE* ON THE WEST COAST OF SOUTH AFRICA

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The abalone *Haliotis midae* forms one of the oldest fisheries on the South African coast, with present-day operations including recreational, subsistence and commercial activities. During the 1990s land-based farming of this species has also been under development and has recently attained commercial scale production. In April 1999 routine monitoring provided evidence of the presence of PSP toxins in cultured abalone. Subsequent analysis of wild abalone collected from the West Coast also revealed the unexpected accumulation of PSP toxins in these non-filter feeding shellfish. Toxicity as measured by the mouse bioassay showed considerable variation between individual animals with maximum values exceeding 1000 μg STXeq 100 g-1. The observation of PSP toxins in abalone coincided spatially and temporally with blooms of the dinoflagellate *Alexandrium catenella*. Toxicity as measured by High Performance Liquid Chromatography was notably higher than that measured by the mouse bioassay. The toxin composition of the abalone was dominated by saxitoxin and therefore differed significantly from the toxin profile of *A. catenella*, indicating either a high capacity for transformation of PSP toxins by abalone or that *A. catenella* was not the source of the toxin. Investigation of the anatomical distribution of toxins revealed that they were not evenly distributed throughout the abalone tissues. The muscular foot, which contributes substantially to the total weight of the soft tissues, and is the organ marketed for human consumption, makes a disproportionately low contribution to the total toxin content of the mollusc. To date, the inability of abalone to detoxify accumulated PSP toxins below the regulatory level threatens the future of the established abalone fishery and the newly developed aquaculture operations on the West Coast.

DETECTION AND IDENTIFICATION OF TOXINS ASSOCIATED WITH A SHELLFISH POISONING INCIDENT IN NEW SOUTH WALES, AUSTRALIA

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In December 1997, 56 people reported gastrointestinal symptoms of either vomiting or diarrhoea after ingestion of pipis (*Plebidonax* sp.). This bivalve mollusc is collected from the intertidal zone of sand on gently sloping ocean beaches, a few centimetres below the surface. Most commercial supplies come from Ballina, New South Wales, which has an annual collection of 300,000 kilograms. Two frozen samples of implicated pipis collected from cases were tested for the presence of toxins. ELISA tests for DSP toxins proved negative, but mouse bioassays were positive, indicating the presence of a lipophilic toxin. Liquid chromatography-mass spectrometry (LC-MS) analyses indicated low levels of okadaic acid, but they were insufficient to account for illnesses. However, other signals were observed that indicated the presence of two possible polyether-like compounds, all with a molecular weight of 876.5, and these were absent in non-toxic samples. Preparative chromatography was performed to isolate these compounds and structural information was generated using tandem and high resolution mass spectrometry and NMR spectroscopy. These compounds were identified as pectenotoxin-2 seco acids, toxins that were reported recently in New Zealand samples of *Dinophysis acuta* and greenshell mussels, although not associated with human illnesses. Examination of toxic samples of pipis revealed the presence of *Dinophysis acuminata* and *D. tripos*.. As LC-MS methods were further developed, a number of related compounds were observed, and these are believed to be metabolites or degradation products of the main compounds.

COMPARISON OF YESSOTOXIN CONCENTRATION IN BLUE MUSSELS (*MYTILUS EDULIS*) BETWEEN COASTAL AND FJORD LOCATIONS IN NORWAY

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Mussels from one coast- and one fjord location in the Sognefjord were sampled (depth 3-5m) every other week during the period March-November 1997. Yessotoxin (YTX) was analysed by HPLC using the fluorogenic reagent DMEQ-TAD for derivatizing of YTX. The mouse bioassay for the diarrhetic shellfish poisoning (DSP) toxins was performed essentially according to the Japanese method with slight modifications including chloroform in the final step of extraction. In addition to okadaic acid and DTX-toxins chloroform extraction includes a wide spectrum of toxins, including pectenotoxins, yessotoxins and among others unknown toxin(s) with neurotoxic potential. The concentration of YTX in mussels determined by HPLC analysis was on average 3 times higher at the location deep in the fjord compared with the coast location. A maximum toxin concentration was observed in May in mussels sampled at the fjord location. The mouse bioassay of most mussel extracts from both fjord and coast location showed high toxicities from April and throughout the sampling period. Most of the samples analysed contained levels of YTX which alone could contribute to the high mouse toxicity of the chloroform extracts. At YTX doses of about 560μg /kg body weight or above, the survival times were in the narrow range of 40-50 minutes. The very short survival times (<30min.) in the mouse bioassay of many of the extracts from the fjord location indicate that the mussels also might contain unknown toxin(s). When survival times were very short, the mice showed PSP-like symptoms, with jumping in the early stages followed by respiratory arrest. Survival times shorter than 40 minutes have not been observed for YTX upon i.p. administration to mice.

SMALL CELL FORMATION IN *DINOPHYSIS* SPP

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Observations of two distinct size classes in natural populations of *Dinophysis* with similar appearance to normal vegetative cells were first reported in *Dinophysis schuettii* (Jorgensen, 1923) and *Dinophysis swezyae* (Taylor, 1976); but Wood (1954), and Norris and Berner (1970) reported intermediate forms exhibiting a continuum between the two extreme sizes. Focused attention on *Dinophysis* spp associated with DSP outbreaks in the last decade has provided new examples of small cells in the genus, sometimes with contours dissimilar from the corresponding vegetative cells; dimorphic individuals; dimorphic populations, and large-small cell couplets. Nevertheless, most studies were hindered by the unavailabilty of cultures or by the usual moderate numbers of field populations of *Dinophysis* spp. Based on in situ observations of concentrated material during intensive samplings for cell cycle studies of *D. acuminata, D. acuta*, and *D. caudata*, and on laboratory incubations of single cell isolations of *D. acuminata* it is documented that: i) During in situ phased cell division, most cells divide normally, but some (1-10%, but occassionally up to 50%) undergo a "depauperating" cellular fission leading to dimorphic cell pairs (one half corresponding to a "normal" cell and the other to a small cell); ii) After separation and sulcal list regeneration, these dimorphic cells become *D. skagii, D. dens* and *D. diegensis*-like individuals, that can also be observed forming dimorphic couplets (large-small cells) attached by their ventral margins; iii) small cells can grow again to normal size, thus explaining observations of thecal intercalary bands, and intermediate forms. Although the sexual nature of the small cells has not been unequivocally demostrated, these observations suggest common patterns in life cycle strategies of *Dinophysis* spp. Intraspecific morphological variability of *Dinophysis* spp in a given geographic area can be largely justified by small cell formation, part of the putative sexual cycle of these species. This once again draws attention to the need for a major revision of the genus.

MITIGATION OF HARMFUL ALGAL BLOOMS IN FISH MARICULTURE

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Harmful algal blooms (HABs) have caused extensive losses at fish mariculture facilities throughout the world. When first experiencing major fish losses, farmers are often ill prepared and are unable to react appropriately or quickly. After recurring HAB-caused fish kills, fish farmers or governments usually begin developing monitoring and mitigation plans of varying complexity and effectiveness.

Experience has shown that there is no single best mitigation method. The simplest, low technology measures are the most desirable, as they the easiest to implement and monitor in the demanding marine environment. Selection of the most suitable techniques should be based on site-specific conditions including the size and volume of culture facilities, water depth and current conditions, vertical distribution of the HAB species, and cause of fish mortality (e.g., neurotoxins, mechanical damage to gills or environmental hypoxia). Towing net-pens away from HABs is a preferred method, if a suitable refuge area is available, but there are significant risks. Isolating net-pen fish from HABs with perimeter skirts or displacing HAB cells from the pens with coarse-bubble aeration or airlift pumping has sometimes been successful. Mariculturists have tried other methods with varying success including sinking of cages, deep pens, pre-emptive harvest, cessation of fish feeding (to lower oxygen use) and culture site relocation. New types of sinkable-offshore pens and enclosed pens with pumped seawater may offer advantages, but are relatively expensive. A promising low-cost technique of apparently little environmental impact is the application of certain types of clay to remove HABs from net-pens by flocculation. I am studying the effect of clay application on benthic invertebrate communities near net pens and the physiological effects of clay on cultured fish.

THE INTEGRATION OF DNA PROBES INTO NEW ZEALAND'S ROUTINE PHYTOPLANKTON MONITORING PROGRAMMES

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New Zealand has established two-tier shellfish biotoxin monitoring programmes for industry and Public Health, with the first tier providing risk assessments for harvesters based on analyses of seawater for toxic microalgae, and the second determining the type and concentration of toxin in shellfish flesh. A drawback to the first tier has been that several toxic microalgal genera include species which are morphologically similar under the light microscope but which have different toxicities. The rapid designation of species by ribosomal RNA targeted DNA probes for the genera *Pseudo-nitzschia* and *Alexandrium* has proved invaluable for rectifying this problem. The probes have now been integrated into the phytoplankton monitoring programmes and whole cell format probes are routinely used for the discrimination of toxic from non-toxic *Pseudo- nitzschia* species, and have been used to differentiate between the toxic *Alexandrium catenella* and morphologically similar strains of non-toxic *A. fraterculus*. Sandwich hybridisation format DNA probe assays (SHA) do not depend on whole cells, and have been used successfully to detect rRNA of *Pseudo-nitzschia* species in seawater following bloom collapse. At that time cells are often absent in samples but rRNA and domoic acid can still be present in bloom debris. SHAs have also been successfully trialled for the detection of raphidophytes in field samples, and will provide finfish farmers with a rapid and cost effective method for monitoring bloom development in the ichthyotoxic *Heterosigma akashiwo*. The SHA may prove more suitable than the whole cell format probes for fragile microalgae such as the raphidophytes and biotoxic *Gymnodinium* species.

WHERE ARE THE HARMFUL ALGAE? THIN LAYERS OF PHYTOPLANKTON, AND THEIR IMPLICATIONS FOR UNDERSTANDING THE DYNAMICS OF HARFUL ALGAL BLOOMS

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In order to conduct field studies on the dynamics of taxa which cause Harmful Algal Blooms, we have to be able to effectively locate the population of interest within the water column. Classical models of phytoplankton ecology assume that cells are reasonably well distributed throughout the upper mixed layer of the sea, or are found in broad, sub-surface chlorophyll maxima. Patterns of distribution occurring at these scales can be studied by collecting samples at depth intervals of ~ 5 or 10 meters. However, this scale may be too coarse to reveal the actual patterns of distribution. As part of an effort to develop instrumentation and sampling methods which increase sampling resolution to centimeter scales, we have conducted seven cruises (1995-1998) in East Sound, in the southern Strait of Georgia near the US/Canadian border. HABs are common in East Sound, and many of the other fjords of this region. Our data shows that broad chlorophyll peaks can sometimes be resolved into a series of individual peaks. At times, phytoplankton may be concentrated into dense, thin layers only 10s of centimeters, to a meter or two thick, with cell concentrations which may be orders of magnitude higher than those of the surrounding water. Frequently, thin layers and fine scale peaks are dominated by a single taxon. We have located populations of HAB organisms, including *Pseudo-nitzschia* spp., *Alexandrium catenella, Dinophysis acuminata, D. norvegica, Chaetoceros concavicornis, Ch. convolutus* and *Noctiluca scintillans* which are restricted to very narrow bands, or thin layers within the water column. Our observations have two important implications for the study of HABs: (1) thin layers of potentially toxic or harmful algae may easily escape detection by routine monitoring programs, (2) thin layers have the potential to result in localized concentrations of toxins much higher than those of the surrounding waters.

PCR-BASED DETECTION ASSAY FOR THE HETEROTROPHIC DINOFLAGELLATE *PFIESTERIA PISCICIDA*

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Since the late summer of 1997, some fish kills in Chesapeake Bay tributaries have been attributed to toxic blooms of *Pfiesteria piscicida*. Because of its complex life cycle, characterized by putative toxic and non-toxic stages, and the presence of other *Pfiesteria*-like dinoflagellate species, the development of specific detection assays is critical to the accurate assessment of *P. piscicida* in the environment. We have partially characterized the non-transcribed spacer (NTS), small subunit (SSU), and internal transcribed spacer (ITS) regions of the rRNA gene cluster from clonally cultured *P. piscicida*, confirmed by SEM plate tabulation. We targeted the NTS and SSU regions for the development of a species-specific PCR-based assay. Primers were designed (two forward primers in the NTS and a reverse primer in the SSU) for two PCR assays yielding amplicons of 311 and 429 bp. Controls for the integrity of the DNA PCR templates were "universal" actin primers (kindly provided by Dr. G.W. Warr, Medical University of SC, USA). The specificity of the PCR-based assay was assessed by testing clonal cultures of *P. piscicida*, three presumptive *P. piscicida* isolates (CCMP 1830, 1831 and 1834), *Cryptoperidiniopsis brodyi, Prorocentrum minimum*, and 21 unidentified CCMP isolates. Only *P. piscicida*, and the presumptive *P. piscicida* CCMP 1830, 1831 and 1834 tested positive. Sequences of all amplicons were identical to that obtained with *P. piscicida*. To validate the application of the PCR-based assay to environmental samples, 61 water samples from Chesapeake Bay tributaries were tested using actin amplification as control for the quality of the DNA template. For the 17 samples that tested positive, amplicon sequences were identical to that of *P. piscicida*. Quantitative assay formats based on the *P. piscicida* NTS/SSU sequences are under development. [Supported by grants NIEHS 1PO1 ES09563, and ECOHAB NA860P0492 (G.R.V.)]

AN ELISA WITH BROAD SPECIFICITY FOR MICROCYSTIN HEPATOTOXINS

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Cyanobacteria (blue-green algae) capable of producing hepatotoxins, e.g. *Microcystis* spp. can be present in fresh water world-wide. These toxins, inhibit ser/thr protein-phosphatases, and induce hepatocyte necrosis/apoptosis. They are toxic to man, other mammals, and to fish-including salmonid species. This group includes the microcystin (MC) heptapeptides (over 50 known variants) and nodularin, a pentapeptide. Antibodies raised against a novel cyanobacterial toxin analogue-cBSA conjugate were used, together with ovalbumin-toxin-conjugates as a plate coater to develop a competitive ELISA. The ELISA is designed to detect most cyanobacterial hepatotoxins with equal sensitivity. It has a detection limit below 0.1 ng/ml, and a limit of quantitation lower than the WHO-proposed guideline (1 μg/l) for drinking water.. Water analyses can be performed without sample pre-concentration The assay shows good cross-reactivity with all microcystin analogues tested to date, and has ~100% cross-reactivity with nodularin (relative to MC-YR). The assay is robust and has been used successfully in the analysis of an array of aqueous matrices, including bloom samples collected from New Zealand lakes and rivers. The broad specificity of the ELISA makes suitable for use as a quick screening procedure for the detection of cyanobacterial hepatotoxins in water and the aquatic food chain.

DOMOIC ACID BINDS IRON: A POSSIBLE ROLE FOR THE TOXIN?

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Toxic species of the pennate diatom *Pseudo-nitzschia* can produce domoic acid, an analog of the excitatory neurotransmitter glutamate and a known causative agent of the human illness amnesic shellfish poisoning. Domoic acid is a small tricarboxylate amino acid whose algal metabolic role is presently unknown. The chemical structure of domoic acid resembles that of iron-complexing agents, suggesting a role for domoic acid as an iron chelator. Certain *Pseudo-nitzschia* species may therefore produce domoic acid to bind iron, possibly increasing the availability of this essential micronutrient. There is increasing evidence that marine phytoplankton can influence ocean productivity by releasing strong, trace metal-binding dissolved organic compounds. Dissolved iron in surface seawater has been found to be >99% bound organically.Using a highly sensitive adsorptive cathodic stripping voltammetric technique, we investigated the iron-binding characteristics of domoic acid (certified reference material, NRC) and found it does chelate iron to such a degree as to significantly affect its chemical speciation (log K with respect to Fe' = 8.7 (+/ 0.5)).Cultures of *Pseudo-nitzschia australis* were grown for the first time in a chemically defined, trace metal clean media. Results show that under iron-stressed conditions ([Fe(III)'] = 10^{-14} M) these organisms produced 7.6 nM of iron-binding chelator with the same conditional stability constant as that measured for the domoic acid standard (log K' = 8.2). This dissolved domoic acid concentration is similar to concentrations produced by *Pseudo-nitzschia* species previously published and is also consistent with that expected during a recent bloom along the California coast in May-June, 1998. The concentration of domoic acid measured in toxic bloom conditions, taken together with the complexing binding strength we have measured will render iron significantly bound to domoic acid. Thus, our findings show that domoic acid may affect the availability of iron. Possible biological roles for domoic acid include acquisition and/or detoxification of trace metals in seawater.

THE USE OF MOLECULAR TECHNIQUES TO CHARACTERISE TOXIC CYANOBACTERIA.

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In recent years there has been an explosion in methods and applications of molecular typing for both prokaryotic and eukaryotic organisms. The advent of DNA amplification techniques using the polymerase chain reaction (PCR) has resulted in gene characterisation as a means of identification becoming technically straightforward and widely applicable. Here we describe several approaches to identifying and characterising toxic cyanobacteria of the genus *Anabaena* and several geographically distinct isolates of *Cylindrospermopsis raciborskii*.

As an alternative to the more commonly studied 16S rRNA gene, we looked at a DNA dependent RNA polymerase gene (rpoC1) which has been described as more discriminatory. Following PCR amplification and gene sequencing we were able to confirm that all our *C. raciborskii* isolates were the same species, although they showed both coiled and straight morphotypes. *C. raciborskii* could be placed in a phylogenetic tree in relation to other cyanobacterial species using rpoC1 sequence data. A *C. raciborskii* specific PCR test targeting the rpoC1 gene was developed and used successfully to identify the organism directly from environmental samples.

A similar analysis permitted taxonomic grouping of members of the genus *Anabaena*. An *Anabaena circinalis* specific PCR was developed which was also successfully applied to environmental samples. *A. circinalis* is the only species of this genus in Australia known to produce saxitoxins (STXs), and approximately 60% of environmental samples containing this organism have been found to be toxic. *A. circinalis* is difficult to distinguish from other members of this genus microscopically, therefore PCR identification is useful as a rapid diagnostic tool. In the case of *C. raciborskii* we have also identified 2 genes, a polyketide synthase (pks) and a peptide synthetase (ps), implicated in toxin production. We have developed a PCR test which will rapidly identify the presence of these genes in samples taken from *C. raciborskii* blooms.

CONFIRMATION OF DUAL MECHANISM OF METHYL INTRODUCTION IN BREVETOXIN BIOSYNTHESIS: COMPREHENSIVE INTERPRETATION OF DINOFLAGELLATE POLYKETIDE BIOSYNTHESIS.

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The polycyclic ethers represented by brevetoxins (BTXs) are unique dinoflagellate metabolites, and the mechanism of their biosynthesis has been of great interest. More than ten years ago, our group and Nakanishi's group independently reported the unusual features of the biosynthesis of these dinoflagellate polyethers. In their reports, it was suggested that brevetoxins are aberrant polyketides, in which methyl side chains are derived from both methionine and acetate. This dual mechanism of methyl introduction has been recently questioned, since similar aberrant dinoflagellate polyketides, okadaic acid derivatives were found to receive their methyl groups exclusively from acetate units, and the pathway was considered to be characteristic of dinoflagellate polyketide biosynthesis. In fact, 13C-labelling studies of BTX biosynthesis accompanied considerable randomization, which left certtain ambiguity. In order to reinvestigate the biosynthesis, we made a large number of new isolates of *Gymnodinium breve* from the existing culture, and selected those isolates which caused the least randomization. Using those strains, we unequivocally confirmed our previous finding; four methyl groups attached to C-8, C-22, C-25 and C-36 in BTX-B are indeed derived from methionine methyl, and the rest from acetate methyl. We also confirmed the previously reported incorporation patterns of labelled acetates, and, in addition, were able to obtain new information regarding the C-C connectivity and incorporation rate. Based on these results, we arrived at a new interpretation of BTX biosynthesis, which also may apply to other dinoflagellate polyketides in general . Supported by NIH grant GM28754.

STRUCTURES AND LC/MS DETERMINATION OF AZASPIRACIDS, CAUSATIVE TOXINS OF AZASPIRACID POISONING

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We isolated and determined the novel structures of the major toxins responsible for a new type of shellfish poisoning occurring in Europe since 1995. Based on the presence of a unique azaspiro ring and a carboxylic acid in the molecules, we named the toxins azaspiracids and proposed to call the poisoning azaspiracid poisoning (AZP). The toxins showed mouse lethalities between 100 and 200 microgram per kilogram body weight and were deduced to have caused in human nausea, vomiting, severe diarrhea, and stomach cramp. In order to determine the toxins in shellfish, we developed a rapid and sensitive method by using liquid chromatography/mass spectrometry (LC/MS). Toxins in mussel meats were cleaned up by simple solid phase extraction and separated on a reversed phase column with a mobile phase containing dilute acetic acid. The method provided a mass detection limit of 50 pg for azaspiracid. The sensitivity was approximately 80000 times greater than the mouse bioassay. The precision in quantification was rigorously tested by using purified standard toxins. The usefulness of the method was verified by applying to the mussels collected at Arranmore Island of Ireland in 1997.

MORTALITY OF SEA LIONS ALONG THE CENTRAL CALIFORNIAN COAST LINKED TO A TOXIC DIATOM BLOOM

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Over 400 California sea lions (*Zalophus californianus)* died and many others displayed signs of neurological dysfunction along the central California coast during May and June 1998. A bloom of *Pseudo-nitzschia australis* was observed in the Monterey Bay region during the same period. This bloom was associated with production of domoic acid (DA) that was also detected in planktivorous fish, including the northern anchovy (*Engraulis mordax*), and in sea lion body fluids. These and other concurrent observations demonstrate the trophic transfer of DA resulting in marine mammal mortalities. In contrast to fish, blue mussels (Mytilus edulus) collected during the DA outbreak contained no or only trace amounts of DA. This presentation summarizes the use of *Pseudo-nitzschia* species-specific DNA probes, scanning electron microscopy, a DA receptor binding assay, and tandem mass-spectrometry to detect the toxic bloom and trophic transfer of the DA. Histopathological analyses of sea lions that suffered acute exposure to DA revealed brain lesions concentrated in the anterior ventral hippocampus. Toxic blooms of *Pseudo-nitzschia* can be expected in the future along the shores of California and elsewhere. The rapid and dramatic nature of the 1998 mortality event suggests that even a short pulse of DA in the food web, lasting only days or weeks in a localized area, may be sufficient to kill or sicken marine wildlife.

LOCALISATION OF PSP TOXINS IN DINOFLAGELLATES OF THE GENUS *ALEXANDRIUM*

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Up to now, less informations of the localization and the origin of toxins are known from HAB causing organisms. Therefore, several toxic dinoflagellates of the genus *Alexandrium (A. lusitanicum, A. tamarense, A. catenella*) with different toxin patterns, detected with HPLC, were used. They were stained for PSP toxins with a polyclonal antibody against neoSTX/GTX and a monoclonal antibody against STX to locate the distribution of toxins inside the dinoflagellate cell or in associated bacteria. Fluorescent labeled secondary antibodies were used to detect the signal with laser scanning microscopy. Different to the distribution of okadaic acid we found PSP toxins located near the cell periphery of the cell concentrated in small patches near the plasmamembrane and the amphiesmal vesicles. Our investigations are showing different affinities of the antibodies to the different species of *Alexandrium* used. Cells of the same culture showing very variable toxin contents probably reflecting their different physiological conditions. Dinoflagellates shading off their thecal plates are showing a labeling at the plasmamembrane of the naked cells and the isolated thecal plates. All cultures we used were not kept under axenic conditions, but cells were not inhabiting intracellular bacteria as we know from our ultrastructural investigations. A localization of the toxins in bacteria present outside the dinoflagellates could not be observed.Supported by the German BMBF, TEPS-Project

THE CONTROL OF HARMFUL ALGAL BLOOMS USING CLAY MINERALS

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This study examined the flocculation and sedimentation of four HAB species *(Gymnodinium breve, Heterosigma akashiwo, Pfisteria piscicida*, and *Aureocuccus anophagefferens*) using a variety of clays. More than 30 clays were tested and these could be grouped into five categories ranging from high to low removal efficiency. Clays that removed one species well were not always effective on other species. 80%-90% cell removal could be achieved with loadings as low as 30 ppm in some cases (e.g. *Gymnodinium and Heterosigma),* but with other species and clays, 250 ppm or higher loadings were needed. Surfactants such as polyaluminum chloride (PAC) reduced the loading needed for cell removal by nearly an order of magnitude. Moreover, cell removal efficiency increased significantly when the clay-cell suspension was agitated (in the case of *Aureococcus*), but removal was less efficient with increasing salinity (in the case of *Pfiesteria*) and higher cell concentration. Experiments in large tanks and settling columns demonstrated that higher amounts of clay than predicted by simple scaling factors from test tube experiments will be necessary to maintain comparable removal rates. Clay additions using phosphatic clay resulted in 80% mortality of precipitated *Gymnodinium* cells at 500 ppm. No cell growth was observed six days after treatment. While clays can both release and remove important algal nutrients (NO3/NO2, NH4, PO4 and SiO4) in the water column, laboratory studies have shown that the release can be moderated considerably by the addition of small amounts of PAC. Lastly, studies on the impact of clays on the benthic and planktonic communities are in progress and will be discussed.

DEGRADATION OF THE CYANOBACTERIAL TOXIN CYLINDROSPERMOPSIN USING VARIOUS TREATMENT METHODS

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Toxin producing cyanobacteria are becoming more abundant in drinking water reservoirs around the World. *Cylindrospermopsis raciborskii* is a potential toxin producing cyanobacterium commonly found in drinking water reservoirs in South East Queensland. This cyanobacterium produces the cyclic hepatotoxin cylindrospermopsin. With potentially lethal effects on humans, cylindrospermopsin must be removed from drinking water. Typical treatment methods (flocculation, sedimentation and filtration) are effective in cellular removal, however, such processes often result in cell lysis and hence the release of intracellular toxins. It is important to remove the dissolved cyanotoxin from the water. Several methods of removal have been examined including, oxidation by chlorine and ozone, and ultra violet degradation with the addition of titanium dioxide. Each method is effective in cylindrospermopsin removal under various conditions. Chlorination is pH dependent and ultra violet degradation is more efficient with the addition of titanium dioxide.

THE CYANOBACTERIAL TOXIN, CYLINDROSPERMOPSIN: HUMAN HEALTH RISK ASSESSMENT

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The toxin, cylindrospermopsin, has been shown to be produced in Australia by the cyanobacteria, *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum*. *A. ovalisporum* is infrequently detected and has thus far only been found in Queensland. By contrast, *C. raciborskii* is widely distributed in freshwater systems in Queensland and to a lesser extent in other states. Concentrations of cylindrospermopsin in water bodies have been shown to exceed 100μg/L under heavy bloom conditions with both organisms. The main toxic mechanism of cylindrospermopsin is believed to be inhibition of protein synthesis. The liver is the main target organ with substantial fatty vacuolation occurring. In addition to liver toxicity, other organs affected include the kidney, thymus and spleen. A thrombohaemorrhagic lesion in the eye orbit is also seen in some dosed animals Studies with radiolabelled cylindrospermopsin has shown that a proportion of the cylindrospermopsin is strongly bound in the liver as a metabolite. Studies using cell lines also indicate that cylindrospermopsin is metabolically activated in order to produce inhibition of protein synthesis. In addition, studies with cylindrospermopsin dosed mice have shown that covalent binding occurs in liver DNA. The acute intraperitoneal 1 day LD50 for cylindrospermopsin in mice is 2mg/kg , while the 5 day LD50 is 0.2mg/kg which demonstrates the time course of cylindrospermopsin intoxication. The acute oral LD50 is approximately 6mg/kg.. In mice dosed daily via drinking water for a period of 90 days, the NOAEL was approximately 0.15mg/kg/day. A risk assessment using suitable safety factors suggests that a guideline value of 15μg/L would apply based on this data. If demonstrated DNA binding was to be considered, and if cylindrospermopsin was classified as a genotoxin, then a guideline value of 1.5μg/L would be applicable. Considering the levels of cylindrospermopsin found in water reservoirs in Queensland, it is essential that suitable treatment techniques be employed to remove this toxin to levels of less than 1μg/L when water is to be used for human consumption.

PFIESTERIA PISCICIDA: IDENTIFYING TOXINS ASSOCIATED WITH A NOVEL HUMAN NEUROTOXIC SYNDROME

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In July and August 1997 disabling neurologic symptoms in watermen occurred along with fish kills in several estuarine rivers along the Chesapeake Bay in Maryland. The skin lesions observed in small fish (menhaden) resembled those reported for years in North Carolina by Burkholder and Noga, who have identified a novel dinoflagellate, *Pfiesteria piscicida*, that appears to be associated with both fish kills and human intoxication. The toxic agent or agents involved in these events are as yet unidentified, and may possess some unusual characteristics as compared to other known algal toxins. We are utilizing several strategies to identify toxin(s) involved in both human and fish toxicity. These include in vitro systems capable of detecting microphysiological responses in cells and neuronal membrane preparations that express ligand binding for multiple neurotransmitter receptors; ex vivo monitoring of regional changes in brain activity and metabolism in fish; and in vivo observation of behavioral and physiological changes in fish. For measuring physiological events in vitro, we have utilized an automated microphysiometer that can detect small changes in extracellular media reflecting cell state. For localizing and quantitating regional brain activation in tilapia, we have developed autoradiographic methods using ^{14}C-deoxyglucose as a marker for cell metabolic activity. For studying toxicity in fish, we have validated monitoring and observation systems.of both larval and adult organisms. We have also developed methods to assess histamine-dependent physiological responses in fish skin cells because of the characteristic dermal lesions reported in fish taken from waters with *Pfiesteria piscicida*. Supported by funds from NIEHS-NIH.

ESTUARINE ALGICIDAL BACTERIA: THEIR DETECTION AND EFFECT ON HARMFUL ALGAL SPECIES

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The Huon Estuary in Tasmania, Australia, flows unregulated through a predominantly native forest catchment. The river has high levels of humics and tannins and contains approximately 15 small aquaculture farms. Over fifty bacterial colonies with differing morphologies were isolated on marine agar from the river and its sediments.The cell free media of six of the bacteria had a powerful lytic effect on the toxic dinoflagellate *Gymnodinium catenatum*, an introduced species which blooms intermittently in the estuary causing shellfish farm closures. Two of the bacteria were identified, based on molecular and phenotypic analyses, as novel *Pseudoalteromonas* species. The remaining algicidal bacteria were identified as *Cytophaga*, *Flavobacterium* and *Bacillis* species. Thirty eight strains of Antarctic seaice bacteria were also tested none of which showed any permanent effect on *Gymnodinium catenatum*. Tests on other algal species were also carried out. The six estuarine bacterial species had no effect on rotifer and diatom species tested. Fatty acid profiles of the fifty bacterial strains and field samples and plate counts reflected great variability indicating the bacterial diversity present in the estuary. This investigation supports the concept that bacteria can play an aggressive role in the microbial food chain. We believe that the potential to use these novel bacteria as agents against toxic bloom-forming microalgae and in other endeavours, will be an area for future research opportunities.

WATERMASS STRATIFICATION AND HARMFUL ALGAL BLOOMS: AN ALTERNATIVE VIEW

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Watermass stratification is classically considered the essential habitat condition needed for dinoflagellates and other flagellate species to bloom. This requirement is thought to reflect their relative inability, unlike diatoms, to tolerate the elevated shear-stress associated with water-column mixing or developing during horizontal transport of local and coastal currents. Populations entrained in such currents are subjected to shear-stress forces considerably greater than those in smaller-scale Langmuir circulation patterns which flagellates exploit to their ecological advantage. This paper analyzes the swimming speeds of 71 dinoflagellate, raphidophyte and other flagellate taxa, and compares these to the turbulence fields developing during representative wind conditions, and to the current velocities reported from frontal zones. The results suggest that the classical stratification-bloom paradigm needs revision. Tolerance of, and exposure to well-mixed watermasses, and/or entrainment within fast-moving current systems appear to be important, even essential, aspects of the bloom ecology of many flagellate species. Dinoflagellate species array along a mixing-stratification gradient, rather than exhibit a uniform response (= association) to the degree of stratification. The cell size of many bloom species falls within the diameters of the physical cells developing during the turbulence cascade which dissipates the energy introduced by wind-induced mixing. This diminishes the potentially damaging impact of associated shear-stress forces. The motility of many species exceeds in situ vertical current velocities allowing diel migrational patterns to persist. The ability of dinoflagellate species to tolerate the vertical mixing of offshore, frontal zones, where abundant populations often develope, suggests that "pelagic seeding zones" occur, from which seed stock is recruited and dispersed, and may be as important as sediment "seed banks" of dinoflagellate resting cysts in providing seed stock, particularly for holoplanktonic species. I suggest that watermass stratification frequently noted to accompany flagellate blooms is often a secondary, parallel event less essential then than some other factor in triggering the observed bloom. Dinoflagellates and other flagellates generally may have evolved a biophysical tolerance to frequent, growth-promoting, water column disturbances, rather than depend exclusively upon the quiescent conditions of a stratified water column whose characteristic nutrient-poor conditions would promote stasis of the population, rather than growth-promotion.

UTILISATION OF PARALYTIC SHELLFISH TOXINS (PST) BY MOLLUSCAN BACTERIA

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Investigations into determining if shellfish bacteria were capable of biotransforming PSTs focussed on several common molluscan species, including: blue mussels, oysters, razorfish, cockles and scallops. Bacteria associated with these shellfish were isolated on marine agar and characterised by Gram stain, colony morphology and carbon utilisation profiles (BiOLOG). Selected isolates from groups demonstrating 90% similarity were screened for their ability to metabolise a range of PSTs (GTX 1/4, GTX2/3, STX, NEO, C1 and B1) using a novel screening method with confirmation by HPLC. Results suggest that bacteria from different shellfish, show varying capacity to utilise and transform PST analogues. For example, isolate M12 (present in mussels) was able to utilise Gonyautoxins (GTX) 1/4 with the concomitant production of GTX 2/3, while strain Q05 (isolated from Queen scallops) apparently degraded GTX 1/4 without the appearance of other GTXs. These findings raise questions as to the possible role of bacteria resident in the shellfish food transport system. Some researchers have suggested that the microflora play a role in supplying nutritional requirements of the host, this study demonstrates that bacteria may also play a part in PST elimination in molluscan species.

BIODEGRADATION OF THE CYANOTOXIN CYLINDROSPERMOPSIN

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Cylindrospermopsin (CYN), a potent hepatotoxin produced by *Cylindrospermopsis raciborskii* has been detected in many drinking water supplies of Queensland. Irrespective whether the toxin occurs in the source water, water resource managers have to provide consumers with drinking water, free from cyanotoxins. Recent and ongoing research has demonstrated that CYN is removed during chemical treatment however some concern remains regarding the formation of potentially harmful disinfection byproducts. Biological degradation is an alternative method of degrading cyanotoxins during pretreatment of drinking water.

The aim of this study was to assess whether bacteria are capable of degrading CYN and even more so to isolate and culture organisms, which have the capacity to degrade CYN in water. The method used was based on a multistep enrichment and isolation technique. A sample of water that had previously demonstrated biodegradative activity for CYN was spiked with an aqueous C. raciborskii cellular extract.The water was then incubated at a constant temperature in the dark. Subsamples were regularly taken and analysed for CYN using HPLC/MS/MS to determine if degradation was occurring. Once log linear degradation was found a subsample was collected and was added to a medium containing purified CYN. The steps were repeated and when during the second run more than 80% of the CYN was degraded the sample was centifuged, supernatant decanted and the pellet washed several times. The cleaned pellet was resuspended and an aliquot added to a fresh medium containing purified CYN. This procedure was repeated several times. On the final enrichment an aliquot of pellet was streaked on to solidified medium containing purified CYN. Single colonies from these plates were transferred back into sterile medium containing purified CYN and toxin concentrations monitored to ensure isolation of CYN degradative bacteria was accomplished.

These bacterial isolates were subjected to numerous analyses to determine their identification. Initial results indicate all degradative orgainsms isolated were gram negative coccobacilli. Sequencing of the genome of these isolates is still in progress.

SIMILAR BLOOMS BUT DIFFERENT RESULTS – A MITIGATION EXPERIENCE

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There have been harmful bloom of *Phaeocystis globosa* in Raoping, on the north-east coast of Guangdong Province, China almost every year in recent years. Two of the biggest blooms happened in October 1997 and the Summer of 1999. In the 1997 bloom, all the fishes and shrimps in about 4,000 culture cages died, million of US dollars of economic losses resulted. In contrast, during the 1999 bloom in the same area and with the same cell density of *Phaeocystis globosa*, all fish farms were towed away from the bayments where the bloom developed and concentrated to the off shore open sea waters, which were not affected by the harmful bloom. Thus, only very small losses were caused by the bloom. The two blooms had two absolutely different results. This provides another good example that movement of cultured stock is a practical and cost effective mitigation for preventing the effects of harmful algal blooms and minimizing fish kills. Several points can be learned from this case study: first, fishmen should have a good knowledge of specific harmful blooms, so that they can take action as soon as the bloom initiated; second, fish pens should be smaller in size and easier to tow, so that they can meet the requirements for fast action and cost effectiveness; and finally, proper and safe refuge areas should be selected.

POTENTIAL PREDATION ON *PFIESTERIA PISCICIDA*

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Laboratory experiments were done to measure the potential grazing pressure of < 200 μm fraction of plankton on *Pfiesteria piscicidia* and to determine which taxa are important predators on this dinoflagellate. During the summer of 1999, water samples were collected from tidal estuaries on the eastern shore of the Chesapeake Bay from which *P. piscicida* has been reported. The water was gently filtered through a 200 μm mesh to remove macrozooplankton. The control treatment was filtered thru a GF/C filter to remove nano- and microzooplankton as well. Non-toxic zoospores (NTZ) of *P. piscicida* (Strain FDEPMDR23, an apparently non-toxic strain) were stained with a vital green fluorescent dye, 5-chloromethylfluorescein diacetate, and added at concentrations of 500 to 1000 cells/ml to the treatments. Number of ingested zoospores per grazer was determined after 10-15 mins. Number of zoospores was enumerated hourly. The dominant micrograzers on *P. piscicida* were certain species of large tintinnid and oligotrich ciliates. Metazoa and non-ciliate protistan taxa were usually minor contributors to grazing by the <200 μm fraction on *P. piscicida.* The instantaneous rate of grazing mortality varied from 0 to ˆ0.46/h (instantaneous rate of growth of NTZ,s varies from ~0 to 0.08/h, depending on irradiance and prey concentration). These results indicate the protistan grazing may, at times, regulate or prevent proliferation of NTZs, but that grazing mortality is highly dependant on species composition as well as abundance of ciliate microzooplankton. Times when grazing pressure are low, in combination with high algal prey densities, may present windows of opportunity for growth of *Pfiesteria* NTZs.

DEPURATION OF DIARRHETIC SHELLFISH TOXINS (DST) FROM MUSSELS, *MYTILUS EDULIS*: NO EVIDENCE THAT FOOD INCREASES THE RATE OF DEPURATION

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The hypothesis that the availability of food increases the rate of depuration of DST in mussels (*Mytilus edulis*) was tested in a laboratory experiment. Mussels naturally containing DST (mean 500 μg OA kg-1 mussel meat) were collected from a mussel farm located on the Swedish west coast during a bloom event. Individual mussels were placed in filtered seawater and given daily rations of a mixture of non-toxic algae as follows: starvation (no food), 0.5% and 1.5% of dry weight body mass day-1. The contents of okadaic acid (OA) and dinophysistoxin-1 (DTX-1) were analyzed using HPLC after 1, 2, 4, 8, 16 and 32 days. Also, the amount of accumulated faeces, total flesh weight and digestive gland weight were measured at the end of each treatment. OA was the major toxin found in all mussels and low amounts of DTX-1 (<10%) were found only in a few specimens. The levels of OA decreased in all treatments with time. In contrast to predictions, levels of toxins were significantly lower in the starvation treatment compared to both food treatments after 32 days. The loss of toxins in the starvation treatment were correlated with a significant loss in the weight of the digestive gland between 16 and 32 days. Only the starvation treatment reached levels of OA below the limit for marketing of mussels at the end of the experiment. We conclude that the mechanisms for depuration of OA from mussels are not associated with turn-over of nutrients and fecal production. In management of toxic mussels, depuration is not likely to be enhanced by adding food to mussels.

ALGAL TOXINS IN MARINE FOOD WEBS

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Herbivorous copepods are key intermediates for the transfer of algal toxins into marine food webs. Toxins from the dinoflagellate *Gymnodinium breve* have been traced through experimental food chains, from copepod grazers to juvenile fish. Field samples taken during a *G. breve* bloom confirm toxin transfer also occurs in natural populations. Brevetoxins (Pb-Tx2 and PbTx-3) were detected in sediment, water column particulates, size fractionated zooplankton, and tissues of fish, sea turtles and marine mammals. A new analytical technique, micellar electrokinetic capillary electrophoresis with laser-induced fluorescence allowed measurements of toxin standards at ~0.10 fg and detection limits in tissue of ~4 pg g-1. In similar experiments, copepods fed on the diatom *Pseudo-nitzschia multiseries* (MU-1) had grazing rates as high as 1,800 cells copepod hr-1 and results of receptor binding assays indicated a range of 3-7 ng domoic acid per copepod within 3 hours.

THE MOST EFFECTIVE AND ENVIRONMENTALLY FRIENDLY WAY OF KILLING ALGAE

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This new invention, called Aquasonic, kills all kinds of algae and provides an everlasting algae prevention system, at the same time restoring the biological balance in the treated water area. The device transmits ultrasonic vibrations through the water, causing the vacuole to tear and the alga to implode, with immediate death as a result. It can handle unto 50 million liters of water using only 15 Watt.There are ultrasonic anti-algae devices for all kinds of water volumes, ranging from 2 meter to 150 meter, larger ranges still being under development.Most algae will be killed within 48 hours. Thread algae may take a couple of weeks, but nevertheless will die.The system is installed by simply plugging the power cable of the electronic part in the mains and floating down the transducer into the water.Since 1997, already 2,000 Aquasonics have been installed in The Netherlands and Belgium, mainly in water reservoirs used by market gardens, where the device has become indispensable.

A BACTERIAL RISK ASSESSMENT AS A MODEL FOR ASSESSING RISKS OF ALGAL BLOOMS

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A risk assessment is a process of determining the probability of occurrence of adverse health effects resulting from exposure to a hazard. There are four steps: 1) hazard identification; 2) hazard characterization; 3) exposure assessment, including a dose-response assessment; and 4) risk characterization. Ideally, for harmful algal blooms, exposure assessments should begin with toxigenic plankton in the sea and end with a probability of illness after ingestion of a seafood containing toxin. Microbiological risk assessments have been developed with models from an animal host through to the consumer, where there is limited information to connect the reservoir with the hazard in the food. One of these is suggested as a model to build upon for algae. The hazard is *Vibrio vulnificus*, a bacterium that grows well in warm seawater. Oysters in the Gulf of Mexico which concentrate this organism through filter feeding have been implicated in illnesses. The assessment considers information from the *Vibrio* in the sea to the ingestion of an oyster meal. A model was developed to consider the prevalence, numbers and seasonality of *V. vulnificus* in oysters, and the influence of meal sizes. From these data, a simulation model, using 10,000 iterations, computed an average probability of illness in a healthy individual of 6.6×10^{-6} from eating a single raw oyster harvested during the warm summer months, and 6.7×10^{-10} in the cooler winter and spring. Assumptions have been made based on existing knowledge and an allowance has been made for uncertainty. The bacterial model would have to be adapted for specific algal situations.

HYDROGEN PEROXIDE INDUCED EFFECTS TOWARD CULTURED CELL LINES: DOES *HETEROSIGMA* PRODUCE TOXIC CONCENTRATIONS?

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The unicellular marine Raphidophyte, *Heterosigma* sp. is associated with coastal fish kills in Japan, New Zealand and Canada. This phytoplankter is common in many coastal waters but is reported to be toxic in specific geographical areas. The mechanism of toxicity is poorly understood and could be due to either the production of a compound similar to brevetoxin or the abnormally high production rates of reactive oxygen species (ROS). One such ROS is hydrogen peroxide (H_2O_2). Production of H_2O_2 is not a constitutive property. The levels of production vary depending on the origin of the isolate, the phase of growth and the environmental conditions for growth. Several of our isolates show rates of production sufficient to achieve an extracellular concentration as high as 5×10^{-7} M. In order to determine if these levels of ROS are sufficient to negatively influence fish, we have perform a series of experiments on isolated cell lines. For comparison, we have examined the sensitivity of a fish gill cell line with the more traditional mouse liver cell line. We have considered if the Heterosigma cells alone or the spent medium from the different cultures grown under different environmental conditions can damage the cell line and if this damage is identical to the destruction due to direct exposure to H_2O_2. Collectively, this data could determine the feasibility of algal-produced H_2O_2 as an ichthyotoxic agent during fish kills.

EXAMPLE OF A *GAMBIERDISCUS TOXICUS* FLARE-UP FOLLOWING THE 1998 CORAL BLEACHING EVENT IN MAYOTTE ISLAND (COMOROS, SOUTH-WEST INDIAN OCEAN)

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The dinoflagellates *Gambierdiscus* spp are distributed worldwide in tropical and subtropical waters and form part of the natural biota of coral reefs. In the Indian ocean, populations have been identified in numerous places including the three species *G. toxicus, G. yasumotoi* and *G. belizeanus. G. toxicus* is the most commonly found and the one recognized to contaminate the food web, inducing ciguatera fish poisoning. From april to august 1998, a severe coral bleaching event occurred in the Comoros archipelago and particularly in Mayotte, affecting almost all the coral species. More than 80% of the bleached corals died. Mass mortality offered new surfaces to multispecific algal turfs colonization which are good environmental conditions for proliferation of epiphytic microalgae such as *Gambierdiscus, Ostreopsis* or *Prorocentrum*. We describe here an unusual large and monospecific bloom of *Gambierdiscus,* which was identified by scanning microscopy as *G. toxicus.* The densities of the dinoflagellate per gram of algae support, which was one year earlier of 384, reached 60,400 in October 1998. This density is the higher ever recorded in the region and one of the highest of the world. Question remains if the global toxic pool of the coral ecosystem may increase seriously for accumulation in the food wed and human poisoning.

DETECTION AND ENUMERATION OF RAPHIDOPHYTES USING RRNA-TARGETED OLIGONUCLEOTIDE PROBES

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We are exploring the development and application of large-subunit rRNA (LSU rRNA)-targeted oligonucleotide probes as tools to aid in the detection and enumeration of the fragile, fish-killing raphidophytes. Oligonucleotide probes potentially specific for *Heterosigma akashiwo* and *Fibrocapsa japonica*, as well as raphidophytes in general, were evaluated using whole cell fluorescent in situ hybridization (FISH). Probes that reacted strongly towards the target organisms and displayed promising specificity were then incorporated into a sandwich hybridization assay (SHA), and evaluated on the basis of their ability to detect and quantify the target organisms in cultured and natural samples. Species-specific SHA,s were successfully developed for both *H. akashiwo* and *F. japonica*, and the sensitivity of these tests is such that resource managers could utilise the assay to detect these species at concentrations well below those that result in fish mortality. Batch culture experiments showed that the response of the SHA towards a constant number of *H. akashiwo* and *F. japonica* cells harvested in exponential versus stationary phase of growth varied by a factor of approximately two, except for dying cells of *H. akashiwo* in late stationary phase. Using the FISH method, cells reacted poorly towards the probes after entering the late stationary phase of growth. The difference between results of the FISH and SHA-based tests suggests that the rRNA in preserved, intact cells is less accessible to the probes than is rRNA released upon cell lysis. The SHA, applied using an automated robotic processor, appears to be a faster, more cost-effective, and easier-to-use method than other cell detection and quantification strategies for *H. akashiwo* and *F. japonica* based on FISH or light microscopy, particularly when large numbers of samples must be processed routinely and rapidly.

LEVELS OF SAXITOXINS ASSOCIATED WITH GROWTH OF THE CYANOBACTERIUM *ANABAENA CIRCINALIS* UNDER VARYING SOURCES AND CONCENTRATIONS OF NITROGEN

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Saxitoxins have been found in Australian populations of the cyanobacterium *Anabaena circinalis*. The C-toxins (C1 and C2) and gonyautoxins (GTX2 and GTX3) are dominant components, while saxitoxin (STX), GTX5 and decarbamoyl gonyautoxins (dcGTX2 and dcGTX3) are minor constituents. Variation in concentration and composition of saxitoxins has been observed in natural populations and cultured strains and may reflect environmental conditions. Laboratory experiments were conducted with a single strain of *A. circinalis* to examine the effect of different nitrogen sources (dissolved atmospheric nitrogen, nitrate or ammonium) and varying concentrations of nitrate (0.0028, 0.28 and 28 mg N L^{-1}) on cyanobacterial growth and levels of saxitoxins. Growth was determined by cell enumeration and concentrations of saxitoxins were analysed by HPLC. All experiments indicated a consistent linear relationship between cell density and concentration of saxitoxins (intracellular + extracellular). Growth of *A. circinalis* was depressed by addition of ammonium (0.04 mg N L^{-1}) and by high levels of nitrate (28 mg N L^{-1}) and these treatments were associated with an increased toxin release. Concentration of extracellular saxitoxins increased with the age of cultures. The composition of intracellular and extracellular toxin profiles were usually similar, however the relative abundance of the different toxins was not always comparable. Extracellular toxin profiles generally comprised a higher proportion of STX and GTX2 and less C1. There was a strong correlation between toxin quota (concentration of saxitoxins per cell) and logarithmic growth rate in three of four experiments.

CLADOPHORA MACROALGAL BLOOMS AT ANAPA (NE BLACK SEA) INTERFERE WITH RECREATION AND TOURISM

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Anapa is the biggest children seaside resort in Russia. In Summer its population (50000) increases 40-60 times. Blooms of the green filamentous algae of genus *Cladophora (C.albida, C.glomerata, C.vagabunda*) were first observed at Anapa coast in early 80s, and during last decade they became annual. The cause of the blooms is the heavy discharge of organic and inorganic nutrients from i) the town sewerage ii) ajacent agricultural fields iii) mountain soil. The vegetation usually starts in February-March and continues through to November; storms interrupt blooms throwing the seaweed on shore. Fine sandy sediments prevent the growth of other macroalgae, whereas *Cladophora* grows unattached, forming tangles that cover up to 100% of the bottom, with the biomass 300-400 gm-2. Due to the very shallow waters at Anapa *Cladophora* is found 800m off shore where depth is not more then 10 m. Alongshore current directed north of Anapa provides the resourse for the bloom 15km off the town where children summer camps situated.Waves drive seaweed to beach, which results in formation of brown gelly mass near surfline; at the central beach of Anapa bulldozers are employed to remove decaying *Cladophora* mass. The bloom became a concern for those involved in touristic business because of the tourists' complaints.

BALLAST WATER EXCHANGE: TESTING THE DILUTION METHOD (PETROBRAS, BRAZIL)

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The dilution method, devised by Petrobras (Brazilian State Oil Company), is the first initiative in Brazil to tackle ballast water management. This method is based on loading the ballast water through the top of the tank and, simultaneously, unloading by gravity through the bottom at the same flow rate. In June 1998, a full scale trial was performed on the oil carrier M/V Lavras (Petrobras) to assess the efficiency of this method on a segregated ballast tank (2,286m^3). The ballast water was taken while the ship was anchored on the coast of Pará State, Brazil (local depth = 15m). The ballast exchange was carried out en route, at 200 n.m. offshore (local depths > 2000m). Characterization of coastal and oceanic waters (controls) was done by casting sampling devices from amidships. A simulation model based on the dilution rate of methylene blue established 8 sampling points in the tank, representing areas of average and low exchange rates. Sampling from the tank was done with a pneumatic pump (20mm-diameter hose, flow = 10L/min) which was efficient for phytoplankton (concentrated in a 20μm mesh), but zooplankton sampling required tows (200μm mesh) through a manhole. Sediment from the empty tank was sampled before and after the experiment. The amount of the original water that remained after exchanging 3 tank volumes (21 hours) depended on the parameter analyzed: chlorophyll a (14%), methylene blue (10%), phytoplankton abundance (4%); only oceanic zooplankton groups with the dominance of oceanic copepods were found, and microalgae cysts/resting spores were close to non detected in the water column. The sediment was not quantified, but visual observation after deballast indicated that the thick layers previously present had been washed. Cysts/resting spores that remained in the tank (1-2 x 10^5 cysts-spores/L) indicate that organisms found in the sediment represent a problem for further investigation.

GYMNODINIUM BREVE IN THE WESTERN GULF OF MEXICO: RESIDENT VERSUS ADVECTED POPULATIONS AS SEED STOCKS FOR BLOOMS

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Gymnodinium breve is a common, nearly annual bloom species along the Florida shelf. In contrast, Texas blooms are highly intermittent although extremely disruptive to both fishing and tourism industries. The source of Texas red tides is unknown, but it is presumed to be of offshore origins. Unlike Florida, the Texas coast does not have the Loop Current as a source of frontal instrusions on the shelf. Another possibility is that resident *G. breve* populations exist in the near shore environment. We have established a sampling program in coastal waters (<15 km) to examine near shore waters for the presence of *G. breve* and related species. Samples were collected twice monthly from 5 stations distributed along the Texas coast. In the first 10 months of 24 total months of sampling (Nov. 1998 to Sept. 1999), no *G. breve* was recorded from the waters inside 15 km. However, *G. mikimotoi, G. sanguineum* and *G.* spp. were recorded at varying concentrations. Regional patterns of chlorophyll and nutrients suggested distinct hydrographic zones along the coast. Since there is no evidence of a resident population, blooms are likely episodic events seeded into the area. A shelf transect noted nutrient rich water sliding onto the shelf as the result of an anticyclonic feature, and could provide both the offshore source waters and intermittent timing associated with the red tide outbreaks. Routine monitoring in the coastal zone may provide little or no warning of impending red tides in these waters.

ECOPHYSIOLOGY OF SOME DINOFLAGELLATES FROM AMBON BAY, INDONESIA

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Four dinoflagellates, *Alexandrium cohorticula, Alexandrium* sp., *Gymnodinum catenatum*, and *Prorocentrum gracile* were isolated from Ambon Bay, a small, semi-enclosed bay on the island of Ambon. The fifth species *Pyrodinium bahamense* var *bahamense* was isolated from Manila Bay in the Philippines. These species were cultured in natural and artificial media and at different irradiances (10 – 400 mol photon m^{-2} s^{-1}). All species grow well in artificial medium. The maximum growth rate for these species ranged from 0.18 – 0.56 d^{-1}. *Prorocentrum gracile* has the highest growth rate (0.56 d^{-1}) when grown in L1 media. When *Pyrodinium bahamense* var *compressum* was grown in medium L1 plus soil extract from the mangrove area in Ambon Bay its growth rate was higher compared to other media. *Gymnodinium catenatum* showed an increase in growth rate when it was cultured in soil-enhanced media. The onset of light saturation (Ik) for all species studied is around 50 mol photon m^{-2} s^{-1}. The Ik for *Alexandrium cohorticula* was 60 mol photon m^{-2} s^{-1}, but this species still grew at irradiances, as low as 10 mol photon m-2 s-1. This study is the first to explore the ecophysiology of dinoflagellates from Indonesia.

SAHARAN DUST AND FLORIDA RED TIDES: THE CYANOPHYTE CONNECTION

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Prediction of the consequences of harmful algal blooms for humans and other vertebrates is constrained by an inadequate understanding of the factors that promote their initiation. An analysis is made of ~3 decades of red tide strandings, associated fish kills, and concomitant dust loadings on the West Florida shelf. The larger summer blooms of a toxic dinoflagellate, *Gymnodinium breve,* appear to be regularly primed by an aeolian supply of nutrients. Wet deposition of Saharan mineral aerosols may alleviate iron limitation of diazotrophic cyanophytes, which in turn fuel the nitrogen economy of red tides in the eastern Gulf of Mexico. Vagaries of the wind-induced circulation and of selective grazing pressure on phytoplankton competitors within phosphorus-replete coastal waters then determine each year the residence times for exposure of *G. breve*- mediated neurotoxins to fish, manatees, and humans along the southeastern United States.

FIRST RECORD OF BLOOMS OF *COCHLODINIUM SP.* CAUSING MORTALITY OF NET-PEN REARED SALMON ON THE WEST COAST OF CANADA

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Blooms of *Cochlodinium* sp., monitored for the first time on the west coast of Vancouver Island from August to October 1999, caused substantial mortality to farmed salmon accounting for economic losses of about $2 million. Cells of the alga were 30-45 μm in length, 20-30 μm in width and had a torsion of 1.8 to 2 turns. Double cells up to 25% of the biomass were common, and apart from the absence of longer chains the anteriorly placed nucleus and numerous golden chloroplasts matched the morphological description of *Cochlodinium polykrikoides* Margalef. A strong diurnal pattern was observed in blooms at farm sites with high cell concentrations overnight at depths to 25 m and thick surface rafts during the day. Surface concentrations of 60,000 cells ml-1 peaked in early September. Fish stopped feeding when cell counts exceeded 500 cells ml-1 in the netpens, and mortality was observed above 2000 cells ml-1. Bioassays in the field with Atlantic salmon, Salmo salar, smolts demonstrated lethality after 120 min exposure and over 90 % mortality after 500 min when cell concentrations varied from 10,800 to 2,700 cells ml-1 as the bloom moved through the test site. Under controlled laboratory conditions *Salmo salar* smolts died within 27 min with exposure to 7,200 cells ml-1, 55 min with 3,400 cells ml-1, and although fish appeared distressed in 1,000 cells ml-1 only 20% died within the 24 h bioassay. Mitigation protocols of netpen enclosures with 15 m deep tarps and upwelling of deep water by aeration proved less effective against *Cochlodinium* than for *Heterosigma carterae*, the major killer of farmed salmon on the west coast of Vancouver Island.

DETOXICATION OF THE CYANOBACTERIAL TOXIN MICROCYSTIN-LR BY AQUATIC ORGANISMS FROM DIFFERENT TROPHIC LEVELS

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The increased abundance of cyanobacteria (blue green algae) in many aquatic ecosytems can be correlated to high nutrient loads. Cyanobacteria are known to produce a wide range of toxic secundary metabolites which have been increasingly recognized as animal and human health hazard. In order to withstand chemical stressors, many organisms developed several detoxication systems. This detoxication metabolism consists of three phases: phase I activation (cytochrome P-450); phase II conjugation (glutathione S-transferase, glucuronosyltransferases, glucosyltransferases) and phase III excretion or deposition. In this study the cyanobacterial toxin microcystin-LR, which is the most common toxin of blue-green algae was investigated. We studied the uptake of microcystin-LR, the effects on detoxication systems and the first step of the detoxication metabolism in several aquatic organisms from various trophic levels, like aquatic plants, invertebrates, and fish. The heptapeptide microcystin-LR was absorbed by all investigated organisms. This uptake led to a dose-dependend elevation of the microsomal and soluble glutathione S-transferases. The in vitro formation of a glutathion-toxin conjugate could be shown by HPLC- and MALDI-TOF analysis resulting in a glutathione conjugate with m/z 1302.79. The detoxication of microcystin-LR was comparable in all investigated organisms starting with this conjugation to glutathione catalyzed by the glutathione S-transferases.

STUDY OF INTOXICATION MECHANISM OF MICROCYSTINS ON FISH

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Blooming of algae in eutrophic waters can result in the production and accumulation of secondary metabolites e.g. okadaic acid class compounds including microcystins. Microcystins occur worlwide, predominantly in freshwater blooms, but have also been reported in brackish and coastal marine waters. In mammalian toxicological studies, the intoxication of rodents exposed to microcystins is characterised by an inhibition of protein phosphatases. In this study, in vitro inhibitory effects of microcystin-LR, -YR, and -RR on protein phosphatases (PP) type 1 and 2A extracted and purified from grass carp (*Ctenopharyngodon idellus*), as well as PP present in crude fish tissue homogenates were investigated. Results showed that microcystins had specific and potent inhibitory activity against fish protein phosphatases. Liver protein phosphatase activity was completely inhibited in fish injected (i.p) wth microcystin-LR, and this was accompanied by a corresponding decrease in the glutathione (GSH) content in the fish liver. When GSH was injected into the fish before microcystin administration , the ultrastructure of the fish liver was less affected by microcystins as compared to fish that were not pretreated with GSH. hepatocytes from fish not preinjected with GSH showed complete dissociation and severe ultrastructural damage, suggesting that GSH could offer protection to the fish hepatocytes against the toxins. The possible role of GSH in microcystin detoxification is discussed.

MODELLING THE POPULATION DYNAMICS OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE* IN THE ESTUARY OF HIROSHIMA BAY, JAPAN

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In Hiroshima Bay, paralytic shellfish poisoning (PSP) caused by the toxic dinoflagellate *Alexandrium tamarense* has become an annual spring event since 1992. The occurrence of PSP is a serious problem in Hiroshima Bay because the production of cultured oysters from the bay is the highest in Japan. In the present study, on the basis of a physical model which gives advection-diffusion field, the population dynamics of *A.tamarense* in the estuarine region of Hiroshima Bay was analyzed using a numerical model which simulates species competition with the non-toxic diatom *Skeletonema costatum* for silicate and phosphate. The Michaelis-Menten equation and the Droop equation were used for nutrient uptake and growth. Grazing by both oysters and zooplankton were also considered in the model. The model reproduced the population dynamics of both *A. tamarense* and *S. costatum* as well as silicate and phosphate concentrations in the seawater. Population size of the bloom seemed to be determined mainly by the balance between the water exchange and the growth rate of swimming cells, not by the germination rate of cysts. On the other hand, the termination of the bloom was found to be strongly affected by the encystment process of *A. tamarense*. Being different from our expectation, grazing processes including the selective feeding by both oysters and zooplankton showed no significant effect on the population dynamics of *A. tamarense*.

STRUCTURAL ELUCIDATION OF TWENTY CIGUATOXIN CONGENERS

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A breakthrough was made in structural determination of ciguatoxins. High energy collision FAB-MS/MS experiments carried out by selecting Na+ adduct ions as parent ions led to elucidating structures of as many as 20 congeners including new precursor toxins. The structures were further confirmed by charge-remote fragmentation FAB-MS/MS spectrometry after introducing a negative charge group to the molecules. The method required only 10 microgram or less of samples and was applicable to mixtures of congeners. The structural information thus obtained gave a new insight into the diverse biochemical modification of toxins, laid basis to monitoring toxins by LC/MS at pg levels, and provided a new strategy on how to apply immunochemical assays. In the presentation emphasis will be laid on the usefulness of the method to elucidate other marine toxins rather than the details of the analytical methods. Dissemination of our results will be encouraging to the scientists who want structural information on samples which are available only in tiny amounts and in impure forms.

SPATIAL AND TEMPORAL DISTRIBUTION OF HABS IN HONG KONG DURING 1983-1998

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HABs have frequently occurred in Hong Kong in the past 20 years. There were total 740 cases of HABs during 1983-1998. We mapped all occurrences of HABs using a GIS system to examine spatial distribution of HABs in the Hong Kong territorial waters during 1983-1998. The mapped spatial distribution of HABs was compared with fish farm zones, distribution of beaches or topographic features. The comparisons revealed that places of HABs occurrences coincided with fish farm zones, public beaches, or shallow water zones (<10 m). Most HABs occurred in sheltered waters such as Tolo Harbour and Porter Shelter to the east of Hong Kong; and >50% of total HABs cases were between Feb and May with over 10% of total each month and the least frequency was during July-November, under 5% each month. The 7 most frequently-occurring species including (in descending order) *Noctiluca scintillans, Gonyaulax polygramma, Skeletonema costatum, Mesodinium rubrum, Prorocentrum minimum, Ceratium furca*, and *Prorocentrum triestinum* were also mapped. The first 4 species were found to occur in wide regions while the next 3 species only occurred in the northeastern waters where oceanic waters dominate most of time. *Noctiluca scintillans* blooms occurred mostly during December to May, and *Gonyaulax polygramma* blooms only between February and May when oceanic conditions prevail. *Skeletonema costatum* blooms were mostly between May and September. Due to rainfall and the Pearl River estuarine plume influences, salinity started to decrease during this period. These spatial and temporal distribution patterns of HABs were believed to be related to environmental factors such as winds, rainfall, temperature, salinity, and nutrients.

PROGRESS OF RED TIDE CONTROL WITH CLAYS

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Removing red tide organisms with clays is a promising method used to control red tide in the world. This article reviewed our study on red tide prevention with clays in the last few years, which mainly included: (1) Coagulation and mechanisms of clays on red tide organisms. The effects of a series of factors, such as clay kinds and concentration, red tide species, pH value etc. on coagulation were investigated. (2) Preparation of modified clays and coagulation mechanisms. A theoretical model based on study on the coagulation of red tide organisms with clays was established, which showed that the surface modification of clays affected the coagulation and was the main way to improve the capability for clays to remove red tide organisms. Several methods were proposed to prepare the modified clays such as adding PACS (polyhydroxy aluminum chloride) in clays, preparation of positively charged clays by ways of insertion and surface adsorption. (3) Study on the kinetics of clays and modified clays removing red tide organisms. A model of the kinetics of red tide organisms coagulation with clays was established, which theoretical addressed the factors affecting the coagulation rate and suggested ways to increase the coagulation rate. The effects of different kinds and concentration of clays, of the second component PACS and MMH (mixed metal layered hydroxide) and of pH on the coagulation rate were examined. (4) Research on other aspects, including the study of main nutrient adsorption on clays in seawater, and impact of clays on growth of red tide organisms and production of algal toxin. Finally, the problems needed to be resolved in the study of red tide prevention with clays were raised and some suggestion was proposed to the future study in this area.

Harmful ALGAL BLOOMS

Ninth Conference
TASMANIA
2000

ABSTRACTS OF POSTERS

RELATIONSHIPS BETWEEN TOXIC PHYTOPLANKTON AND ENVIRONMENTAL FACTORS IN FISHING HARBORS IN LEBANESE WATERS

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Previous studies showed that harbors are suitable for development of toxic algae and could be a factor of their transport to other places. For the purpose of studying this possibility, samples collected bi-monthly from mid July 1997 to mid June 1998 from 2 harbors and a control station were analysed for the occurence of toxic and potentially toxic algae in relation to environmental factors.The results showed that nutrients are higher in harbor stations and showed great fluctuations.Also the percentage of toxic algae relative to total phytoplankton populations were higher and occured particularly during warm seasons. The following species, which produce potent toxins that can find their way through the food chain to humans, were relatively abundant:*Alexandrium minutum* occured in the period between July and September with maximum concentration of 50000 cells/L in July. *Amphidinium carterae* was present only in autumn with maximum concentration of 2673 cells/L in mid September. *Gyrodinium* cf.*galatheanum* was present in only one harbor in summer time with maximum at the end of July of more than 90000cells/L and absent in winter time. *Pseudo-nitzschia pseudodelicatissima* was only abundant in spring.Other species was also present but in small number such as: *Alexandrium tamarense, Prorocentrum lima, P.minimum, P. mexicanum* and *Ostreopsis siamensis.* Other species harmful to fish and invertebrates such as *Gymnodinium mikimotoi* and *Chaetoceros* spp. were also present.

IS THE ATTACHMENT OF *GAMBIERDISCUS TOXICUS* TO ALGAE IN REEFS IN MONSOON AREAS BIOLOGICALLY OR PHYSICALLY DRIVEN?

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In 1997 and 1998, ciguatera cases were reported in Sibuyan Island Philippines and confirmed by the Philippine Department of Health. In response to this, sampling sites for *Gambierdiscus toxicus* were established in three areas with varying dominant macroalgal species last August – October 1999. The sampling sites were located in Cajidiocan- San Fernando, Sibuyan Island with the following dominant algal species : a) nearshore intertidal zone – *Sargassum polycystum* b) offshore coral reef – *Actinotrichia* spp. c) offshore reef area – *Gelidiella* spp. The G. toxicus cells were observed from algal samples taken from offshore coral reefs while *G. toxicus* were absent from algal thalli from nearshore area. The cell densities in *Actniotrichia* and *Gellidiela thalli* varied from nil to 7.0 cell/sq cm. The suspension of silt from the main Sibuyan Island during heavy and frequent rainfall in southwest monsoon months most likely influenced the marked distribution of *G. toxicus* in the surrounding coastal waters.

PREPARATION OF *DINOPHYSIS* DNA FOR MOLECULAR BIOLOGY APPLICATIONS

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Dinoflagellates of the genus *Dinophysis* are agents of Diarrhoeic Shellfish Poisoning (DSP) and are one of the main risk factors affecting shellfish exploitation in the Iberian Peninsula and Europe. Not much has been learned about the biochemistry, genetics and ecology of these algae because they are notoriously difficult to culture and remain largely recalcitrant to most contemporaneous protocols routinely used with other microbes. Also the methodologies for their detection and identification in natural habitats need to be improved. Both aspects are interrelated since for instance as better knowledge of their genetic will help to develop better methods of detection, and better methods of detections would help the study of their physiology and ecology. At present, rapid progress of knowledge of these species at the molecular and biochemical levels is dependent on technological innovations. Although, some techniques can be directly translated from existing protocols, many of them will require extensive modification. For some molecular biology applications, the preparation of clean DNA is required, however the existing protocols for this purpose need to start with a high amounts of cells. This is particularly difficult to achieve in the case of *Dinophysis* species since cells have to be manually collected from the sea samples. We will present a methodology that allow the obtention of clean enough DNA for molecular biology applications using just a few cells.

MICROCYSTIS AERUGINOSA BLOOM AND THE OCCURRENCE OF MICROCYSTIN IN FRESHWATER POND IN BANGLADESH.

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A bloom of *Microcystis aeruginosa* was occurred in a freshwater pond at Matlab in Chandpur district. Bloom sample was collected and filtered through a glass fiber filter. Methanol-water extract of *M. aeruginosa* was analyzed by high performance liquid chromatography with UV detection detected four types of microcystin viz., Microcystin-RR, Microcystin-YR, Microcystin-LR and Microcystin-LA and those were confirmed by HPLC-MS. Other types of microcystin remains to be identified. Acetic acid extract of bloom sample was analyzed by Theilert HPLC method showed no paralytic shellfish poison in *M.aeruginosa*

PARALYTIC SHELLFISH POISON IN FRESHWATER PUFFER FISH (*TETRAODON CUTCUTIA*) FROM THE RIVER BURIGONGA, BANGLADESH.

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Freshwater puffer fish *Tetraodon cutcutia* was collected from the river Burigonga on 26 February 1999. Acetic acid extracts of skin, muscle and liver were applied to high performance liquid chromatography. Fluorescence detector HPLC demonstrated that the puffer fish contained four types of paralytic shellfish poison vi., sanitarian, decorators, gonyautoxin-4 and decarbamoylgonyautoxin-3. No N-sulfocarbamoyl toxin was detected from hydrolysis sample of acetic acid extract of *T. cutcutia.*

PHYTOPLANKTON DYNAMICS AT A LONG-TERM COASTAL STATION OFF SYDNEY, AUSTRALIA

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Phytoplankton assemblages and their physicochemical environment were investigated during 1997-98 at a marine long-term coastal monitoring station off Sydney, Australia, and compared to those previously seen at this location. Phytoplankton blooms (significant population increases) coincided with episodic slope water intrusions (upwelling/uplifting) lasting 2-22 days and occurring from September to February. These blooms appeared to occur in response to slope water intrusions irrespective of this location's proximity to other major nutrient sources. The hydrological forcing variables of bottom- and surface-water nutrients and temperature, (including time-lagged data), were identified using Principal Component Analysis as those variables that explained 60% of the variability of the total phytoplankton biomass throughout the year. Phytoplankton blooms of similar frequency and magnitude to those seen in this study have been previously recorded. However, in contrast to earlier work, where a variety of taxa dominated throughout the year, the small diatom Thalassiosira partheneia generally dominated blooms in this study. In addition, presence/absence data for the heterotrophic dinoflagellate, *Noctiluca scintillans*, indicated a higher frequency of occurrence for this species than previously documented. *N. scintillans* was observed in 61% of samples collected throughout the year, being absent from only a few samples in the autumn and winter months. It is hypothesised that the shift in dominance from previously recorded bloom species to *Thalassiosira* could be a contributing factor to the increase in *N. scintillans* (a favoured food source by *N. scintillans*) in NSW coastal waters. The reason for the recent dominance of these particular phytoplankters is unclear but may be related to physicochemical conditions such as a decrease in phosphate and oxidised nitrogen concentrations and warmer water temperatures experienced during our sampling period compared to previous years. This includes what appears to be the first record of the potentially toxic diatom *Pseudo-nitzschia australis* in Australian waters and the first record of the harmless diatom *Pleurosigma chilensis* since its "type" description from the coastal waters of Southern Chile, 1941.

A GUIDE TO THE PRONUNCIATION OF THE SCIENTIFIC NAMES FOR HARMFUL ALGAE

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A historical review of scientific nomenclature and the pronunciation of classical languages suggests that there is no objectively correct way to enunciate the technical terms applied to toxic algae. Any guide to pronunciation is always relative to some group of speakers; scientific nomenclature is an artificial construct without a population of normative speakers, living or dead, to whom the bewildered enunciator can have reference. Thus a key to the pronunciation of the Latin and Greek scientific terms in all disciplines, and a fortiori to the pronunciation of those terms applied to harmful algae, must be based on rules of common sense, mutual forbearance, and general intelligibility. This article concludes with a pronouncing guide for the names used in the study of toxic algae based on these principles.

A COMPARISON OF ZOOPLANKTON COMMUNITY STRUCTURE WITHIN AND OUTSIDE THE FIRST RECORDED TOXIC ALGAL BLOOM IN KUWAIT WATERS

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Zooplankton community structure was investigated within and outside the first recorded toxic algal bloom in Kuwait waters. Surface tows were conducted in both areas using a half meter net with 110 micron mesh fitted with a flowmeter. In addition, 150 liters of surface water was filtered through 20 micron mesh. One preserved net tow and filtered surface water sample was analyzed from each location. Net samples were size fractionated by gently wet sieving through Nitex screens to produce the following nominal size classes: >2, 1-2, 0.5-1, 0.2-0.5 and 0.11-0.2mm. The filtered water samples were fractionated to produce >110, 64-110 and 20-64 micron size fractions. Biomass was determined by gently blotting each fraction then weighing to nearest mg. Total biomass was higher outside the patch than within it (1.122 and 0.660 g/m3; out vs. in, respectively) attributed mainly to the <0.5mm fractions. However, biomass was three times greater inside the patch for the 0.5-0.2mm fraction (0.976 and 0.343 g/m3; in vs. out, respectively) and was attributed to the much higher abundance of the copepod *Acartia pacifica* within the bloom. Animals larger than 2mm were found only outside the patch. Total biomass was similar both in and out of the bloom for the filtered surface water samples. However, wet weight was greater out of the patch for the >110 micron fraction (0.118 and 0.083 g/m3; out vs. in, respectively) and lower in the 64 to 110 micron fraction (0.075 and 0.057 g/m3; in vs. out, respectively). The most significant difference in community structure was the absence of fish eggs and near absence of ichthyoplankton within the bloom. Only one fish larvae (0.08 individuals/m3) was found in the sample from within the patch as opposed to an estimated 94 individuals/m3 from outside it. The dominant animals in the various fractions are listed and differences in community structure noted.

POTENTIALLY HARMFUL PHYTOPLANKTON SPECIES FROM QATARI WATERS

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A preliminary survey of potentially toxic phytoplankton species was carried out in Qatari waters between 1993 and 1997. About 46 taxa were recorded during this survey of which dinoflagellates were the most abundant (25 species). Bacillariophyceae were represented by 17 species and Cyanophyceae (blue-green algae) by 4 species, among which *Trichodesmium* was most common. These preliminary results are promising for the practical monitoring of fish toxicity in the region.

DID ALGAL BLOOMS CAUSE FISH KILLS OFF KUWAIT, ARABIAN GULF?

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Kuwait Bay being shallow and eutrophic can sustain rich phytoplankton growth. The Bay ecosystem is severely stressed mostly due to urbanization, shipping, and dual-purpose desalination power plants. Hydrographical conditions (1995/96) in the surface waters show averages ranging from: temperature 14.20 – 30.23 deg. C, 34.80-40.70 psu, oxygen 2.06-6.21 ml/l, and nutrients (μg-at/l) 0-3.21, NH3-N, 0.06-9.03 NO3-N, 0.01-1.15 PO4-P, 9.45-36.26 SiO3-Si. Phytoplankton biomass ranged between 1.81-18.91 μg Chl a/l with 3 ug Chl a/l during August to October. However, during September and October 1999, although the hydrographical conditions were seasonal, thirty tonnes of surface feeding wild mullets and 150 tonnes of caged sea bream died. Out side the cages there were broken patches (10 m x 3 m) of discoloration caused by the benign diatoms *Chaetoceros curvisetus*, *Nitzschia longissima*, and the ciliate *Mesodinium rubrum*. Some of the patches had 3 to 15 million cells/l of the dinoflagellate *Gymnodinium* sp., suspected toxigenic, and yielded chlorophyll a concentrations from 10 to 265 μg Chl a/l. Confirmatory data on the identity of the suspect toxigenic organisms, nature of the toxin, bioassay, total quantity of toxin delivered, not available, but are crucial before implicating the ephemeral algal patches as causative agents of fish kills. Of interest is a mauve colored patch of unknown origin 500m wide, 1m thick and 5km long with no abnormal levels of phytoplankton. Gills of cultured sea bream yielded levels (μg g-1) of As (20-24), Fe (274-987), Ni (7-13.5), Cu (18-31) and Zn (45-252) much higher than the corresponding 4, 36, 1.6, 1.9, and 15 in the gills of wild sea bream. The Se:Ni, Fe:As, Fe:Ni, Zn:Pb ratios in the gills were higher than those in wild fish. The plausible compounding role of these metallic elements causing mass mortality of fish is discussed.

BIOTOXINS VERSUS HARMFUL ALGAE

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During the summer of 1996 samples were collected in an ancient water reservoir. This small, water reservoir of Roman origin, situated in the Guadiana River Basin, is current being used for recreational purposes. Chemical and physical parameters, phytoplankton and zooplankton were determined and organic microcontaminants and cyanobacterial biotoxins were quantified.The High Performance Liquid Cromatography (HPLC)technique was employed for the separation and identification of the differnt microcystins (microcystin-LR, microcystin-RR and microcystin-YR)and nodularins. The results of these studies show that the trophic state of the water reservoir was meso-eutrophy as far as total phosphorus concentration, total nitrogen and chlorophyll *a* concentration.The phytoplankton was dominated by potentially toxic and hepatotoxic cyanobacteria (colonies of *Microcystis* spp. and also *Anabaena* spp and *Aphanizomenon* spp.) The remainig 1% of the phytoplankton was represented by *Staurastrum* spp., *Cryptomonas* spp. and some pennate diatoms. In the zooplankton, ciliates dominated.The microcystin concentration found varied from 0.667-5.000 micrograms per liter for microcystin-LR, 0.252-0.930 micrograms per liter for microcystin-RR and non detected-1.510 micrograms per liter for microcystin-YR.

PSEUDO-NITZSCHIA MULTISERIES IN CULTURE WITH NITRATE AND AMMONIUM AS NITROGEN SOURCES

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The domoic-acid-producing diatom *Pseudo-nitzschia multiseries*, responsible for Amnesic Shellfish Poisoning (ASP), was grown in batch culture with nitrogen supplied as either nitrate (1200 μM and 245 μM) or ammonium (245 μM), providing determined N:P:Si ratios. Cell yield at stationary phase was similar with each of the nitrogen forms and concentrations because Si, rather than N, became limiting. At 245 μM nitrate or ammonium, the cellular domoic acid (DA) concentration (pg/cell) during stationary phase was about five-fold less than that observed at 1200 μM nitrate, indicating a direct proportion to the amount of N added. Changes in the concentration and pattern of production of eight other amino acids were also followed. Production of cysteine, taurine, histidine, aspartic acid, glutamine and glutamic acid peaked during the exponential growth phase and reached low levels during early stationary phase. Proline and alanine showed a more complex pattern, with peaks during early and late stationary phase, respectively, and their cellular levels were also lower than all other amino acids. Histidine was found at the highest concentrations, followed by DA. Changes in the pattern of proline production during the exponential and stationary phases were most consistent with those of DA, suggesting it is involved in the pathway of DA biosynthesis.

HARMFUL ALGAL BLOOMS IN MALAYSIA: REVISITING KIMANIS BAY

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Harmful algal blooms in Malaysia occur only in the South China Sea in the coastal waters of west Sabah, where the causative organism is the dinoflagellate, *Pyrodinium bahamense* var *compressum*. Blooms occur periodically every year, when the dinoflagellate produces paralytic shellfish toxins (PSP) in shellfish, eventuating symptoms of paralyses and deaths in humans since 1976. Long-term studies were carried out intensively in the Kimanis Bay, Sipitang, one of the areas where the blooms were first reported in 1976 and have been recurring frequently since then. The main objectives of this study were to understand the bloom dynamics of the *Pyrodinium* red tides and to determine factors which trigger the bloom events. Data for 1997-99 indicated that *Pyrodinium* blooms occurred frequently in the Kimanis Bay, but were dominated by an almost yearly major bloom event, resulting in the ban of shellfish consumption. An increase in nutrients (mainly NO3-N and PO4-P) concentrations in the water which occurred after rainfall episodes were found to initiate blooms of *Pyrodinium bahamense* var *compressum*, however continuous rainfall did not sustain the blooms due to a decrease in the salinity of the water. Salinity and temperature did not contribute to the initiation of blooms. The long-term nature of the study also allowed the documentation of the effects of El Nino on the bloom events which were experienced in Sabah in 1998. The results of this study and an assessment of the landuse activities in the Sipitang area, which affects the nutrient loading of rivers emptying into the Kimanis Bay, forms the basis of recommendations for the management of *Pyrodinium* red tides in Sabah.

THE RELATIONSHIP BETWEEN PSP TOXIN PRODUCED BY *PYRODINIUM BAHAMENSE* VAR *COMPRESSUM* IN SHELLFISH AND THE PLANKTON IN SABAH, MALAYSIA

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Harmful algal blooms in Malaysia occur only in Sabah, East Malaysia, which is caused by the dinoflagellate *Pyrodinium bahamense* var *compressum*. Monitoring of paralytic shellfish toxins (PSP) is carried out by the Fisheries Department Sabah and is based on shellfish samples collected in the infested area using the mouse-bioassay technique of toxin analyses. Often the detection of the toxin in the shellfish occurs long after the bloom of the dinoflagellate in the plankton. There have also been reports of PSP poisoning occurring even when cell densities of the dinoflagellate are low. A study to determine the relationship between the densities of the dinoflagellate in the plankton and the shellfish as well as between the PSP concentrations in the shellfish and plankton was initated in a long-term study. Results of the study in 1997-98 indicated that cell densities of the dinoflagellate in the shellfish occurred simultaneously with blooms in the water column. High densities of the dinoflagellate in the shellfish occurred between August – October 1997, where a maximum of 4293 (365 cells /cm in the shellfish coincided with a dinoflagellate density of 1870 (47 cells/ L in the plankton. Densities in the shellfish remained high up to 3 months after the bloom of the vegetative cells in the water column, suggesting the possibility of alternate sources of the PSP. However, analyses of the toxin in the planktonic dinoflagellate indicated a high correlation between the density of cells in the water column and the toxin analysed from the plankton. The results of this study provide valuable information towards the development of a model for the prediction of bloom events and its dynamics.

APPLICATION OF THE MOUSE NEUROBLASTOMA (MNB) ASSAY TO THE STUDY OF PSP TOXINS FROM MARINE BACTERIA, DINOFLAGELLATES AND CYANOBACTERIA; A COMPARISON OF DATA GENERATED BY THE MNB ASSAY TO PRE AND POST COLUMN HPLC

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Paralytic shellfish poisoning (PSP) toxins are potent neurotoxins and are produced primarily by dinoflagellates. Additionally, production of these toxins by some cyanobacteria species has been confirmed whereas, the ability of marine bacteria to produce PSP toxins is more controversial. A mouse neuroblastoma assay was developed for the detection of sodium channel blocking toxins such as PSP and has previously been used for analysis of bacteria and shellfish. In this study we have used the mouse neuroblastoma (MNB) assay for the screening and quantification of the sodium channel blocking (SCB) toxins in dinoflagellates, cyanobacteria and marine bacteria from Portuguese waters. This is the first report of use of the MNB technique to detect PSP toxins in the freshwater environment and in evaluating the toxicity of *Gymnodinium catenatum* extracts. Comparison of the results to HPLC data from pre and postcolumn analysis will be presented. Available information suggests that the MNB asssay accurately reflects the total toxicity of samples as calculated from toxin profiles obtained by HPLC.

ENVIRONMENTAL CONDITIONS DURING THE *CHATTONELLA* BLOOM IN THE NORTH SEA AND SKAGERRAK IN MAY 1998

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The high concentration of *Chattonella* was first observed in late April 1998 outside Hirtshals at the Danish side of the Skagerrak and spread to the western coast of Sweden and the Norwegian Skagerrak coast. This was the first time *Chattonella* was registered in Europe, and also the first time it had appeared in high concentrations which resulted in the death of fish. Early in May 1998 the flagellate *Chattonella* caused death in fish farms in an area close the southern tip of Norway. After that incident *Chattonella* disappeared, and was not observed blooming along the Norwegian coast due to changes in the Skagerrak circulation. However at this point in time *Chattonella* was observed at very high concentrations along the north and west coast of Denmark from the Jammerbugt to Esbjerg resulting in death garfish, herring and sandeel. It was feared that a new wave of *Chattonella* may follow the prevailing cyclonic circulation in Skagerrak and reappear at the Norwegian coast. Due to the potential danger to fish farming the situation was closely monitored and in addition to in situ measurements from ships and satellite data, an operational ecological model (NORWECOM) was used in this effort. Great amount of human made nitrate from the south part of the North Sea has probably stimulated the algae bloom.

LABORATORY AND FIELD PSP INTOXICATIONS OF GREEN MUSSELS, *PERNA VIRIDIS* BY *PYRODINIUM BAHAMENSE* VAR. *COMPRESSUM* CELLS

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Paralytic Shellfish Poisoning (PSP) intoxications of green mussels, *Perna viridis* exposed to *Pyrodinium bahamense* var. *compressum* cells under natural and laboratory conditions were studied. Commercial-sized (9-10 cm) shellstocks of green mussels harvested from Bacoor, Cavite, Philippines on September -December 1997 were exposed to F/2 monoalgal culture of the toxic dinoflagellate using several test feeding protocols. The theoretical *P. bahamense* var. *compressum* cell uptake by the green mussels, and PSP toxicities in mussel meats were determined by standard mouse bioassay and high performance liquid chromatography (HPLC) with fluorescence detection. The test feeding protocol with 6h feeding interval using log phase *P. bahamense* var. *compressum* cultured cells at concentrations ranging from 10^5-10^6 cells/L was able to effect cell density uptakes in the mussel meats at concentrations ranging from 10^3-10^5 cells/g. The mouse bioassay results showed toxicities of mussel meats to be >20 μSTX/100g just after 3d exposure using this test feeding protocol. Total toxicities based on HPLC results were significantly higher than those obtained in mouse bioassay. The toxin profile of laboratory (artificially) contaminated *P. viridis* showed the presence of PSP toxins STX, dcSTX, and B1. These toxins were also isolated in naturally (field) contaminated green mussels harvested from Limay, Bataan on May-June 1998 in addition to neoSTX and B2 toxins. The relative tissue distribution of PSP toxins in naturally-contaminated green mussels showed the following trend: adductor mussel > digestive tissues and gonads > gills and mantle > foot.

THE ORIGIN OF THE PERIDININ DINOFLAGELLATE PLASTID

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The typical dinoflagellate chloroplast contains the carotenoid peridinin, which is bound in a unique water soluble light harvesting protein, the extrinsic peridinin chlorophyll protein. These organisms also use a proteobacterial form II RuBisCO. Nuclear phylogenies based on SSU rRNA show dinoflagellates with and without plastids to be related in a manner which implies that the peridinin plastid may have been acquired early in dinoflagellate evolution and lost multiple times. To determine the origin of the peridinin dinoflagellate plastid we have sequenced the large subunit ribosomal RNA and the psbB gene from the plastids of several dinoflagellates. Unfortunately, because the peridinin plastid seems to lack a large master genome, there is no phylogenetic information available from comparitive gene order or content. Despite the high rate of sequence evolution, sequence analyses are the most promising source of phylogenetic information. Analyses of the LSU sequences group dinoflagellate plastids with the plastids of the nonphotosynthetic parasitic apicomplexans, but the high substitution rate makes these phylogenies suspect. Analyses using the psbB gene indicate that the dinoflagellate plastid is derived from the red algal plastid lineage, with moderate support. The dinoflagellate plastid appears to be a secondary, tertiary (or quaternary) endosymbiont which has undergone a dramatic series of changes. We present several acquisition scenarios which attempt to explain these data.

HUMAN EXPOSURE TO CYANOBACTERIAL TOXINS IN DRINKING WATER: PLANS TO EVALUATE RISK OF EXPOSURE FROM PUBLIC WATER SYSTEMS

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There are a number of reports indicating that exposure to the toxins produced by cyanobacteria (blue-green algae) can cause acute or chronic illnesses in humans and other animals. The illnesses attributed to exposure to these toxins include gastroenteritis, respiratory and neurologic effects, skin irritation, allergic responses, and liver damage. The potential routes of exposure include skin contact, inhalation, water and food consumption, and hemodialysis. Although the evidence from reports of human health effects, in conjunction with data from laboratory animal research, suggests that cyanobacterial toxins are responsible for a range of health effects, there have been few epidemiologic studies of these associations. In addition, it is unclear which of the possible pathways of exposure are most important. For example, the extent of human exposure through drinking water is unknown but may be extensive because conventional drinking water treatment does not remove these toxins. The objective of our proposed study is to assess the public health impact of exposure to cyanobacterial toxins present in drinking water. First, we plan to assess the extent of potential human exposure by identifying public water systems where cyanobacterial blooms are likely to occur in the source water. We will collect water samples from source water, finished drinking water as it enters the distribution system, and tap water in homes (without point-of-use water filtration devices) and analyze the samples for cyanobacterial toxins. If we find measurable levels of these toxins in water samples from finished drinking water and household taps, we will develop a protocol to assess the human health effects potentially associated with this exposure.

WHAT IS THE RESPIRATORY IRRITANT IN THE AIR DURING FLORIDA RED TIDES?

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Florida red tide is one of the most notorious of all Harmful Algal Blooms (HABs), occurring on a virtually annual basis along Florida's Gulf coast. The incriminated dinoflagellate, *Gymnodinium breve*, causes major epizootics, produces toxic shellfish when the dinoflagellate is filter-accumulated in clams and oysters, and releases an irritating toxicant into the air when surf conditions are turbulent. Brevetoxins are neurotoxins, they inhibit cell-mediated immunological processes, and they are potent bronchoconstrictors. Very little is known about the toxicological sequelae that accompany human respiratory exposure to toxins in seaspray and wind-dried toxic particles of *G. breve*. Radioimmuno-,enzyme-linked immuno- and immuno-fluorescent assays have provided a qualitative measure of toxin accumulated in cells, tissues, and other biological materials derived from red tide exposure. Development of a quantitative "biomarker of exposure" is presented. The research describes, in the lab and field, the physical and chemical character, and concentration of toxins in wind and seaspray. Uptake, distribution, metabolism, and clearance of labeled brevetoxin in animal models is also presented. Using a sheep model investigation of airway contractility in response to brevetoxin challenge, dose response, drug intervention, mast cell involvement, and synergy with existing asthma or hyperactive airway syndromes is being used to correlate human symptoms with animals signs. We also collect human throat and nasal swabs to measure brevetoxin. Biomarker data is being collected in conjunction with epidemiologic and clinical data, to better define the acute and chronic effects of exposure. The research overall will contribute to the model of animal and human health effects of aerosolized red tide toxins.

A MOLECULAR ANALYSIS OF CYANOBACTERIAL BLOOM DYNAMICS

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Bloom-forming toxigenic cyanobacteria occur worldwide and some species such as *Microcystis aeruginosa* and *Anabaena circinalis*, are a particular problem in Australia. There appears to be variation in the strains of known toxigenic species in various regions. One recent study has shown that genetic variation of strains within one region may be limited *(Nodularia* in Australia, Bolch et al. 1999). However, between regions, other studies show genetic divergence of strains (Neilan et al. 1995, Bolch et al. 1996, Neilan et al. 1997). This study focuses on a molecular analysis of cyanobacterial blooms at one site, in the New England region of Australia. Genetic variation was determined by examination of the intergenic spacer region (IGS) of the b and a-phycocyanin subunits. We have shown that it is possible to routinely obtain template for DNA amplification (PCR), and differentiate the strains in bloom samples. Differentiation was either by restriction endonuclease digestion of the IGS (restriction fragment length polymorphism [RFLP]) or sequencing. Different strains of the one morphotype have been identified within the one site.

THE FIRST TOXICOLOGICAL STUDIES OF HARMFUL ALGAL BLOOMS IN LATVIAN WATERS

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The presented paper could be considered as the first attempt to determine the toxicity of potentially toxic cyanobacteria in Latvian water bodies used as sources for drinking water, for fisheries and recreation. Occurrence of potentially toxic species, their toxicity and toxin content of 37 bloom samples were examined in the southern part of the Gulf of Riga (Eastern Baltic Sea), in the River Daugava and in two eutrophic lakes (Lielais Baltezers and Mazais Baltezers; their canals and infiltration basins). The River Daugava and the Lake Mazais Baltezers play a significant role in drinking water supply of Riga, the capital of Latvia. The toxicity of *Nodularia spumigena* blooms in the Gulf of Riga was compared with toxicity detected in the Open Baltic Sea. Sixteen strains of potentially toxic cyanobacteria (*Microcystis aeruginosa, M. wesenbergii, Nodularia spumigena)* representing 6 collection sites were also isolated from the Gulf of Riga and toxicity under controlled laboratory conditions was investigated. Toxicological studies were carried out by applying bioassay experiments with *Artemia salina* as well as by PP1 assays and HPLC. Toxin analyses were performed at the University of Dundee and Fridrich – Schiller – University of Jena.Ecological and toxicological examination of summer blooms in Latvian waters revealed the abundance of toxic cyanobacteria. Studies of occurrence and toxicity of harmful phytoplankton showed that in Latvian inland waters (in eutrophic lakes Mazais Baltezers and Lielais Baltezers and in the river Daugava) and in the coastal zone of the Gulf of Riga the main attention have to be paid to the bloom events of *Microcystis* spp., whereas in the Open part of the Gulf of Riga – to the bloom of *Nodularia spumigena*. Microcystins were detected in 92 % of freshwater samples and nodularin – in 43 % of the Gulf of Riga samples. All cultured strains isolated from the Gulf of Riga contained toxins

SCANNING ELECTRON MICROSCOPY OF SEXUAL STAGES OF THE PENNATE DIATOM *PSEUDO-NITZSCHIA MULTISERIES* (HASLE) HASLE

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The fine structure and ontogeny of sexual stages from cultures of the domoic-acid-producing dioecious pennate diatom *Pseudo-nitzschia multiseries* were examined by scanning electron microscopy. The structure of gametangia, position of the gametes relative to the gametangia, and their sequential maturation observed previously using light microscopy were confirmed in our material. As well, the general structure of the auxospore conforms to the pattern of development in many previously investigated pennate diatoms. Scales were observed in the gamete wall as well as in the internal lining of the primary wall of the auxospore. Scales in gamete walls were not found previously in any diatom, while in the primary auxospore wall they were known only in three other species. The presence of scales in sexual stages of the morphologically advanced diatom *P. multiseries* supports the monophyletic origin of diatoms and their relationship to the scale-bearing ancestors. The initial cell divides once while still within the auxospore. It may thus exit by piercing the apex of the perizonium when one of the sibling cells slides along the other during the first vegetative division. Initial epivalves show several morphological irregularities compared to typical vegetative valves; the initial hypovalve may bear a more typical morphology. Finally, we discovered a correlation between the molecular (ssu tRNA) phylogeny of diatoms and the basic types of auxospores. The lack of similar reports from other species may be attributed to the scarcity of investigations into the fine structure of sexual cells in diatoms. Similarly, scales may be found more commonly when other species are carefully examined. Then, gamete and auxospore structure may prove useful in defining higher rank taxa in the natural phylogeny of diatoms.

TOXIC AND POTENTIALLY TOXIC DINOFLAGELLATES FROM THE MEXICAN CARIBBEAN SEA.

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Some species of the dinoflagellate genera *Dinophysis* and *Prorocentrum* can produce powerful toxins that affect human health by consumtion of poisoned shellfish (Diarrhetic Shellfish Poisoning, DSP), whereas *Gambierdiscus toxicus* has been associated with Ciguatera fishfood poisoning, in tropical areas. In the Mexican Caribbean, there is no taxonomic study of phytoplankton and benthic microalgae. Material recently collected from various points along the coasts of the Mexican Caribbean: plankton by net (54 μm), sediment and epiphytes from large macroalgae and other plants, was used to study the toxic, potentially toxic and associated dinoflagellates. We recorded the presence of known toxic species: *Dinophysis hastata, D. rotundata, Gambierdiscus toxicus, G. yasumotoi, Prorocentrum lima*, and *P. mexicanum,* plus other associated species: *Gambierdiscus belizeanus, Prorocentrum hoffmanianum* and *Pyrodinium bahamense* var. *bahamense*. Some of these species are truly planktonic forms: *Dinophysis* spp. and *P. bahamense* var. *bahamense*, whereas other are benthic and epiphyte forms: *G. belizeanus, G. toxicus, G. yasumotoi, P. lima, P. hoffmanianum* and other *Prorocentrum* species, all distributed in shallow coastal areas and coastal lagoons. The three species described for the genus *Gambierdiscus* are found in the study area. Most of the species have been studied by light and scanning electron microscopy. We have not made studies to detect toxins production of the dinoflagellates recorded. No cases of Ciguatera or DSP have been properly documented in detail in this region, although unofficial reports point to several cases of Ciguatera by comsumption of fish.

THE MOLECULAR DIVERSITY OF *ANABAENA CIRCINALIS*

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The cyanobacteria is a diverse bacterial phylum with respect to form, function and habitat. Members of the genus *Anabaena* are a major cause of freshwater noxious cyanobacterial blooms, which have a broad geographical distribution. Strains identified as *Anabaena circinalis* have been found to produce a range of neurotoxins including, anatoxin-a, anatoxin-a(s) and the paralytic shellfish poisons (PSPs). Interestingly, only Australian isolates of *A. circinalis* produce the PSPs, while North American, European and Asian isolates exclusively produce the anatoxins. The PSPs consist of the parent compound saxitoxin and its derivatives, which result in paralysis via the blocking of neuronal sodium channels.

Primary structure analysis of the 16S rRNA gene was performed for 50 strains of the cyanobacterial order Nostocales. The phylogenies presented illustrate the evolutionary affiliations of *A. circinalis*, and other PSP producing cyanobacteria. A cluster of 21 strains, including Australian PSP neurotoxic isolates identified by morphology as *A. circinalis*, form a monophyletic group. However, the *Anabaena* appears to be a polyphyletic and contains strains clustering within the genus *Nostoc*. In addition, genus-specific sequences were used to design primers permitting identification of PSP producing cyanobacteria via DNA amplification.

IN VIVO DETERMINATION OF ALGAL PIGMENTS BY FLUOSPECTROSCOPIC MEASUREMENT

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Phytoplankters in marine and fresh waters comprise of a diverse assemblage of pro- and eucaryotic autotrophic microalgae. The qualitative and quantitative assessment of this important algal association, which is responsible for approximately half of the plant production on earth, is still a challenge to limnologists and oceanographers.The 'spectral groups' of phytoplankton (green, blue, brown, mixed) areeach characterised by a specific compo-sition of photosynthetic pigments and, consequently, by a specific excitation spectrum of the chlorophyll-fluorescence. In a new approach we demonstrate that it is possible to calculate the their respective biovolume of four spectral groups of phytoplankton from fluorescence excitation spectra recorded with a newly-developed probe. This is a submersible fluorometer which measures the emission intensity for excitation in five character-istic wavelength ranges employing pulsed light-emitting diodes. The five-point excitation spectra (5 wavelength ranges) are deconvoluted on the basis of norm spectra which have been obtained by analysis of several species of each spectral group. Results of measurements carried out in lakes of Northern Germany recorded with the probe are presented. The possible detection of harmful algal bloom developments is discussed.

EFFECTS OF INCREASED NUTRIENTS ON CIGUATERA ASSOCIATED DINOFLAGELLATES OF THE GREAT BARRIER REEF

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Anthropogenic land development adjacent to the Great Barrier Reef (GBR) has been implicated in increasing levels of sediment, humus and nutrients being discharged downstream, thus increasing the fertility of the water column and lagoon sediments. We investigated the effects of elevated nutrient (nitrogen: N, phosphorus: P) levels on ciguatera associated dinoflagellates: *Gambierdiscus toxicus, Prorocentrum* spp., *Ostreopsis* spp., *Coolia monotis, Sinophysis* spp., *Amphidinium* spp., *Scrippsiella* spp. and *Gymnodinium* spp., on host brown algae *Chnoospora implexa, Sargassum* spp., *Colpomenia sinuosa* and reef flat sediments of Heron Island. Total dinoflagellate abundances within fertilised aquaria increased by 25%, 35% and 64% on *Chnoospora implexa, Sargassum* spp. and *Colpomenia sinuosa*, respectively. *Prorocentrum* spp. constituted up to 60% of the total dinoflagellate population of which *P. mexicanum* was the dominant species. On the reef flat, total dinoflagellate abundances were an order of magnitude lower than aquaria although fertilisation increased total dinoflagellates abundance by 8% and 19% on *Chnoospora implexa* and *Sargassum* spp., respectively and decreased by 45% on *Colpomenia sinuosa*. *Prorocentrum* spp. constituting 46 % of the total population of which *P. lima* was the dominant species. Total dinoflagellate abundance increased by 72% in the enriched sediments of the reef flat and by 58 % in the aquaria sediments. *Prorocentrum lima* and *P. mexicanum* are highly sensitive ($P<0.01$) to elevated sediment nutrients. Increased downstream and point source nutrients from anthropogenic and agricultural developments into the ecosystems of the Great Barrier reef may ultimately be a direct link to additional outbreaks of ciguatera.

ENDOSYMBIOSIS: DINOFLAGELLATES WITH FUCOXANTHINS

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Colourless dinoflagellates may establish endosymbiotic relationships with photosynthetic algae on different levels of organisation. These range from undefined numbers of endosymbionts in *Noctiluca*, binucleated stages with morphologically preserved endosymbionts in *Peridinium foliaceum* and *P. balticum* to a mononucleated subcellular structure. In the last case the endosymbiotic origin of the chloroplasts is revealed by a class-deviating pigmentation. A scheme involving subcellular organisation is presented to illustrate key steps in the establishment of endosymbiosis. Since chloroplast capture by horizontal evolution is a large evolutionary step, increased emphasis on photosynthetic capability when delimitating taxonomic entities should be considered. One group of dinoflagellates, including the toxic species *Gymnodinium galatheanum, Gym. breve* and *Gyrodinium aff. aureolum*, contains fucoxanthin and its two 19'-acyloxyderivatives as main carotenoids. A detailed spectrometric reinvestigation of *Gym. galatheanum*, including 1H-NMR and CD, showed the 3 fucoxanthins to possess the same chiral structures as in other algal classes. Some minor xanthophylls are structurally related to pigments first isolated as animal metabolic products in the marine food chain, including 3 new esters related to halocynthiaxanthin. A hypothesis evaluating the possibility of joint host/endosymbiont interaction in carotenoid synthesis is discussed in terms of the endosymbiont theory. The carotenoids of *Gym. galatheanum* and *Pelagomonas calceolata* are compared. Guided by the taxonomic distribution pattern of fucoxanthins and gyroxanthin diester, our earlier hypothesis on a prymnesiophycean origin of the chloroplasts is extended to also include a possible pelagophycean origin.

DOMOIC ACID DEPURATION IN THE MUSSEL *MYTILUS GALLOPROVINCIALIS*: EFFECTS OF SIZE TEMPERATURE AND SALINITY.

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Raft mussels *Mytilus galloprovincialis* which had incorporated domoic acid during a *Pseudonitzschia* bloom, were collected from the Galician Rías and placed at temperatures of 18 and 22 C and salinities of 12.5 and 31 PSU. Samples were taken at the start of the experiment and daily during the four subsequent days and the domoic acid contents of the soft tissues were analysed. As a first approximation a one-compartment model was fitted to the data, using logarithmically transformed data and a linear regression fit, with good quantitative but with a slightly deficient qualitative results due. No of the three factors checked nor their interactions had significant effect on depuration rate, partially because of the large interindividual variation. A two-compartment model described qualitatively better the depuration but its contribution was not statistically significant. The parameters of the model obtained by least squares minimization of the residuals suggest the possibility of a small second compartment of very small or null depuration rate, as detected in other species.

RAPHIDOPHYCEAE IN DUTCH COASTAL WATERS: ENVIRONMENTAL CONTROL OF NEUROTOXICITY

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Raphidophyceae have been recorded yearly in north-western Europe since 1989; the number of observations has increased steadily. At least 4 species have been recognised in Dutch coastal waters: *Chattonella antiqua, Chattonella marina, Fibrocapsa japonica* and *Heterosigma akashiwo*. Occasionally small blooms of Raphidophytes have been recorded in this region, most recently in July/August of 1998 when large numbers (> 10^4 cells/l) of *Fibrocapsa japonica* were observed.Raphidophyte species are known to produce toxins, whereas unfavourable environmental conditions induce cyst formation. So far, cysts of Raphidophytes have not been reported in the southern North Sea. For most European strains of Raphidophytes toxin synthesis have not been analysed. Preliminary studies on *Fibrocapsa japonica*, grown under different ratios of nitrate and phosphate, showed that both at low nitrate and phosphate concentrations had a short period of growth. Toxin concentrations appeared to be highest when growth ceased especially after phosphate became limiting. The toxin profiles changed during the age of the cultures, while most components diminished when cultures declined. Toxicity tests on the individually sampled compounds are yet to be performed. For further research it is very important to determine the set of environmental conditions that favours bloom formation. Complementary field studies will be conducted to test the relevance of our laboratory experiments.

TOXIC GONYAULAX *GRINDLEY REINECKE* IN THE NORTH-WESTERN ADRIATIC SEA (ITALY)

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Different phytoplankton related phenomena such as red tides, mucilage and algal toxins affected the north-western Adriatic sea. This caused a deep concern because of the importance of this area for tourist presence and for being the main Italian area producing edible shellfish, mostly blue mussels (*Mytilus galloprovincialis*). DSP due to Adriatic blue mussel ingestion has occurred since the end of the 1980s and the toxins have been originated from *Dinophysis* spp. Severe economic consequence took place because of closing shellfish farms for long period. Edible shellfish have been always investigated for toxicity (DSP, PSP, ASP) and water monitored for the presence of harmful algae. During summer 1997 unusual toxicity has been detected, by mouse bioassay, in blue mussels from shellfish farms along the coast of Emilia-Romagna. Phytoplankton analyses revealed the presence of different potentially toxic species among which *Gonyaulax grindley* Reinecke (=*Protoceratium reticulatum* (Claparhde & Lachmann) Buetschli), never before recognized as toxic alga in the Adriatic sea. Unusual toxicity led us to perform test on mussels and the presence of high level of yessotoxin and derivatives has been detected. The same toxicity appeared in summer 1998 and 1999. *G. grindley* was found from June to September raising low cell number (1000 cell/L in 1998 and 560 in 1999). Following the monitoring observations, G. grindley Adriatic strain has been isolated and cultivated. The cultures have been carried out using f/2 culture medium at different dilutions. The algal growth was measured by counting cells every other day and the algae for toxin analyses were collected at different growth phases, exponential, early stationary and stationary. The toxins have been detected by HPLC following Yasumoto e Takizawa (1997) method. In comparison with the New Zealand *G. grindley* strain, very high level of yessotoxin have been detected in the Adriatic strain: 5.83 and 10.04 pg/cell at 13th and 20th growth day respectively instead of ca 3 pg/cell.

HORIZONTAL AND VERTICAL DISTRIBUTION *OF PYRODINIUM BAHAMENSE* CYSTS IN SEDIMENTS OF MALAMPAYA SOUND, PALAWAN, PHILIPPINES

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The cysts of *Pyrodinium bahamense* var. *compressum* were investigated in the inner and outer sounds of Malampaya, Palawan to determine the variation in its distribution and abundance. The intent is also to map out potential seed bed areas for possible bloom initiation. The vertical profile of *P. bahamense* cysts, however, were also examined to determine its distribution and to know how deep they can concentrate and penetrate in the sediment. For horizontal distribution, twenty six (26) sampling stations were sampled during northeast monsoon, southwest monsoon and tradewinds. Four (4) stations were also sampled for vertical distibution profile, two stations both for the inner and outer sounds,respectively. For three seasons, it was observed that relatively high concentration of *P. bahamense* cysts were consistently found in the surface layer (1-2 cm) of the sediment particularly in stations located in the northwestern part of the outer sound. Relatively few cysts were obsered in the inner sound of Malampaya for three consecutive sampling periods. During the NE Monsoon the concentration of *P. bahamense* cysts ranged from 0 to 254 cysts cm3 while during the SW monsoon, the concentration ranged from 0 to 191 cysts cm3. The highest cyst concentration which ranged from 0 to 421 cysts cm3 was recorded during the tradewinds. For vertical profile, *P. bahamense* cysts were found deposited in the deeper sediment of the northwestern part of the outer sound. Cysts were found up to 9-10 cm depth with the highest concentration of 153 cysts cm3 at 5-6 cm depth of the sediment core. No cysts was deposited in the deeper sediment of the inner sound of Malampaya. Results of the study showed that *P. bahamense* cysts were concentrated and widely distributed in the northwestern part of the outer sound of Malampaya and can be considered as potential "seed bed" area for future blooms. The green mussel (*Perna viridis)* farms are all located in the shallow coastal embayment of the inner sound. Although, there are no shellfish farm available in the outer sound, this suggests that the *P. bahamense* will have the tendency to recur in the area and the bloom has the possibility to be carried by wind-driven current into the inner sound. Thus, effective monitoring and management is necessary to prevent PSP outbreaks in the area.

PHYSICAL PROCESSES CONTROL THE DISTRIBUTION OF LIGHT, NUTRIENTS AND CYANOBACTERIA IN A LARGE TROPICAL REGULATED RIVER

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The Fitzroy River is a typical regulated turbid river in Queensland, Australia where the construction of a barrage for water supply purposes has created a 50 km long slender lake with a maximum depth of 12m. Monsoonal rainfall predominantly from December to March produces large flows transporting large amounts of sediments. For the rest of the year flows are negligible and the barrage is subject to frequent blue-green algal problems.Turbidity and nutrients in the river are always high after a flood. As the flow recedes the turbidity declines slowly due to settling of fine particles taking months for the water to clear. Nutrient concentrations also decline before the onset of algal growth. Temperature stratification sets in in spring and strongly influences the light climate, the oxygen distribution, nutrient release at the sediment/water interface and the succession of cyanobacteria from large to small species. Despite persistent temperature stratification and nutrient concentrations in the epilimnion below the limit of detection, cyanobacterial blooms persist for months. We attribute their success to the intermittent strong wind forcing enabling enhanced nutrient transfer across the metalimnion. Algal growth is sustained until the barrage is flushed with the next flood.

AN INVESTIGATION OF THE IDENTITY AND POTENTIAL TOXICITY OF GYMNODINIOID SPECIES PRESENT IN FALSE BAY, SOUTH AFRICA

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In 1989 a toxic *Gymnodinium* species was responsible for abalone (*Haliotis midae*) mortalities and other sub- and intertidal marine fauna in False Bay, on the south coast of South Africa. In 1995, this species was also responsible for larval mortalities in land-based abalone farms. Attempts to isolate and culture this species have revealed the presence of several Gymnodinioid species on the south coast, a number of which have been successfully isolated and cultured. The identity of these dinoflagellates have been investigated by examining the external morphology and cellular pigment composition. These studies, have to date, revealed the presence of seven different species, one of which closely resembles *Gymnodinium mikimotoi.* Other species identified include *G. sanguineum, G. pyrenoidosum, G. pulchellum, Gyrodinium* cf. *corsicum* and *Lepidodinium viride.* Another species remains unidentified. The toxicity of each culture and its filtrate was ascertained by means of an *Artemia* bioassay (ARTOXKIT), a routinely used method in marine and aquatic toxicology. A similar experimental procedure to the *Artemia* bioassay was used to investigate the toxicity of these species on both abalone larvae and spat (3mm animals). For comparative purposes similar experiments were conducted on *G. mikimotoi* (Isolation site: Australia), *G. aureolum* (Isolation site: Norway) and *G. breve* (Isolation site: Florida).

FEEDING BEHAVIOR OF INDIVIDUALS AND GROUPS OF KING SCALLOPS *(PECTEN MAXIMUS*) CONTAMINATED EXPERIMENTALLY WITH PSP AND DETOXIFIED

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The French king scallop (*Pecten maximus*) is commonly harvested in Northern Brittany near coastal areas where summer blooms of the toxic dinoflagellate *Alexandrium minutum* usually occur. An experimental recirculating flume was used to study PSP contamination and detoxification patterns in scallops fed *A. minutum* and then non-toxic flagellates. Experiments were performed simultaneously with a set of five isolated individuals compared to a control (classical evaluation of ecophysiological parameters) and with two groups of 30 animals placed in a 100-L raceway (biodeposits method).At the individual level, the linear relationship between particulate organic matter (POM) and measurement of mean STX eq. per cell allowed comparison of real and calculated toxin absorption rates. Observations of the feeding physiology of scallop groups were subsequently analyzed using a general linear method describing the effects of the shift in food supply as well as the trend in the parameter studied when the diet was changed. Isolated scallops showed considerable interindividual variation in FTA values despite identical feeding conditions. Yet, regardless of the feeding activity of each individual, toxin uptake in tissues was always less than release in feces. Toxin absorption rates varied with time from one individual to another, but were always distributed in tissues in the following order: digestive gland > kidneys > other tissues. Mean bioaccumulation efficiency (Be) in tissues reached 17% for an absorption efficiency (Ae) of 43%. For both groups, feeding behavior at the time of the shift to a non-toxic diet changed drastically depending on the algal species used to detoxify the scallops. A diet based on *Tetraselmis suecica* appeared to stimulate biodeposition, clearance and filtration, whereas one based on *Isochrysis galbana* had the opposite effect. During detoxification periods following initial toxicity of either 150 or 350 µg STX.eq./100 g of meat, an average of more than two weeks was needed for toxin levels in scallops to reach the quarantine threshold. Toxin analysis in tissues also showed obvious STX neoformation in kidneys.

PHYSICAL AND BIOLOGICAL FACTORS ASSOCIATED WITH TEMPORAL AND SPATIAL DISTRIBUTION OF *PFIESTERIA PISCICIDA* IN THE CHESAPEAKE BAY

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Pfiesteria piscicida, a heterotrophic dinoflagellate, has been associated with adverse fish and human health effects in estuarine environments extending from Delaware to Florida. Due to public concern over the risk of exposure to putative toxins and the economic loss related to fish kills in the Chesapeake Bay in Maryland, the Maryland Department of Natural Resources and collaborating laboratories began monitoring various tributaries of the Bay in 1997 and has developed those efforts into an extensive and comprehensive program for 1999. Targeted rivers include those in which *Pfiesteria piscicida* has been previously detected (through molecular methods, scanning electron microscopy and fish kill bioassays), rivers having a possible predisposition to *Pfiesteria* activity (similar chemical and physical characteristics as affected systems), and rivers with no history or apparent tendency for *Pfiesteria*-related problems. A PCR based primer-probe system for the detection of the organism has been developed by our laboratory and extensively tested against various dinoflagellate cultures for specificity. In 1999, *Pfiesteria* piscicida was not detectable in surface waters through the spring and early summer, first appearing in selected rivers in July and in multiple sites during August and September. Similar observations from this region in 1998 suggest that environmental conditions in the Chesapeake favor population blooms for this organism in the late summer and fall. Mid-winter sediment sampling (and dinoflagellate culture) in the same region has demonstrated the widespread distribution of the organism in this region. An analysis of biological and physicochemical parameters associated with the detection of *Pfiesteria piscicida* and assessment of those parameters in waters consistently negative for the organism will enable us to identify conditions conducive to *Pfiesteria* blooms. Hypotheses generated with these data will be tested through ongoing longitudinal analyses of samples collected at multiple stations throughout this region.

MONOCLONAL ANTIBODY-BASED ENZYME IMMUNOASSAY FOR DOMOIC ACID BY USING HAPTEN-PROTEIN CONJUGATES OBTAINED AT THE NANOMOLAR LEVEL IN A REVERSED MICELLAR MEDIUM.

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A competitive enzyme-linked immunosorbent assay (ELISA) to measure domoic acid (DA) has been developed. DA-protein conjugates were prepared via the mixed anhydride method of Erlanger performed in a reversed micellar medium using 0.32-0.64 μmol of DA in a 100-fold molar excess of protein. Two specific monoclonal antibodies (MAbs), 1D12 and 3E1 were produced by hybridoma technology following immunization of a BALB/c mouse with a DA-bovine serum albumin conjugate. No significant cross-reactivity was observed with either glutamic, aspartic or kainic acids. Using MAb 1D12, matrix effect was investigated by comparing standard calibration curve obtained in diluent buffer with DA recovery from spiked mussel extracts (crude or pre-cleaned through a solid phase extraction column). No significant matrix effect was observed for the pre-cleaned extracts (r2 = 0.96), whereas the use of crude extracts caused negative interferences (i.e. DA is less available for antibody interaction). The working range achieved with this ELISA (0.03 – 0.3 μg/g of original mussel tissue) strongly suggests its potential as alternative assay for routine monitoring of shellfish.

QUANTIFICATION OF *ALEXANDRIUM TAMARENSE* BY FLOW CYTOMETRY AND IN SITU-HYBRIDISATION FOR MONITORING HARMFUL ALGAL BLOOMS

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During several cruises with the research vessel HEINCKE (BAH/AWI, Helgoland, Germany) we were able to detect different species of dinoflagellates – causing algal blooms – by flow cytometry. In May 1997 and May 1998 we found toxic algal blooms at the Eastcoast of Scotland and at the Orkney Islands. These blooms were caused by a saxitoxin producing species of *Alexandrium tamarense*. We detected these dinoflagellates in water samples by flow cytometry and in situ-hybridisation of the 18SrRNA and the 28SrRNA. The population of *Alexandrium tamarense* in multiparametric dot-plots can be correlated with the saxitoxin concentration measured by HPLC. We were also able to show a correlation between cell concentration measured by flow cytometry and the cell concentration determined by the microscopic method of Utermöhl. The flow cytometric data (list-mode) were also analysed automatically by a trained neural network (backpropagation) and compared with results from conventional gating, corresponding populations in 2D-dot-plots.Our measurements and results of different dinoflagellates from algal blooms clearly show that it is possible to detect specific algae species and subspecies by in situ-hybridisation with specific oligonucleotide probes and to quantify it in a flow cytometer. In combination with the automatic recognition of the dinoflagellates by a neural network this system is a further step towards automated plankton monitoring. (Supported by the German BMBF, TEPS-Project)

FIRST ECOLOGICAL AND TOXICOLOGICAL STUDIES ON *CYLINDROSPERMOPSIS RACIBORSKII* (CYANOBACTERIA) IN FRANCE

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Cylindrospermopsis raciborskii, a blooming species of cyanobacteria (blue-green algae) was first identified in France in 1994 from a fishing pond localized in the south Paris area (Viry-Châtillon). The geographic distribution of this species first recorded in tropical to subtropical areas (Indonesia, 1913, India, 1912, Australia,1979), is now spread to temperate countries, and this french localization is among the most septentrional ones. In november 1979, an outbreak of severe hepatoenterities and renal damage involved 148 people among an Aboriginal population in Palm Island (North Queensland, Australia). A new toxin, cylindrospermopsin, was purified from a *C. raciborskii* strain, hold responsible for the disease. Then other toxicity episodes due to *C. raciborskii* were observed in Australian freshwater reservoirs and recreational waters. More recently, paralytic shellfish poisons (PSPs) were identified in two Brazilian *C. raciborskii* strains. Public health problem raised by *C. raciborskii* proliferations led us to develop a monitoring program on the occurrence of *C. raciborskii* in Viry-Châtillon pond. Since july 1998 and every month, the variations of phytoplanktonic populations were studied and the physical and chemical parameters measured. After a positive mouse bioassay, bloom extracts were screened systematically for cylindrospermopsin and for PSPs, toxins potentially produced by *C. raciborskii* strains: cylindrospermopsin was analyzed by High Performance Liquid Chromatography coupled to a ultra-violet diode-array detector (HPLC-DAD), and PSPs by both the mouse neuroblastoma cell bioassay and HPLC with postcolumm oxidation followed by fluorescence detection.This monitoring program allowed the determination of *C. raciborskii* proliferation conditions, and the characterization of its in situ toxic potential. Besides, laboratory cultures have been carried out to determine taxonomical, physiological and toxicological properties of the strains isolated from blooms.

POLYCLONAL ANTIBODIES AGAINST YESSOTOXIN: TOOLS FOR SHELLFISH TOXIN RESEARCH

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Polyclonal antibodies that bind to yessotoxin (YTX) and its analogs, members of the diarrhetic shellfish poisoning (DSP) toxin group, have been produced. The antibodies were used to develop an ELISA as part of a research program carried out developing a screening system for shellfish toxins. The system includes an array of ELISAs to detect toxins in each of the four toxin groups. The ELISA for YTX has a working range of 30-1000 pg/mL and can be used for quantifying toxin in shellfish and algal extracts. The use of these antibodies and ELISAs in other research applications is now being investigated. Because of their broad specificity, the YTX antibodies bind to other structurally-similar antigens. This property, together with the sensitivity of the ELISA method, is being used as a tool for identifying new compounds that are unrecognized by existing analytical procedures. Algal extracts were fractionated by HPLC and the fractions analyzed by ELISA. Some of the immunoreactive fractions did not correspond with known YTX compounds.Antibodies were also used to prepare immunoaffinity matrices. Immunoaffinity chromatography provided a simple concentration and cleanup step of toxin in samples of interest. We also anticipate that our antibodies will bind the fluorescent DMEQ-TAD derivatives of YTX that are currently used for HPLC analyses of YTX, and we are currently exploring ways to exploit this property.

DEVELOPMENT OF CLADE (*ROSEOBACTER* AND *ALTEROMONAS*) AND SPECIES-SPECIFIC OLIGONUCLEOTIDE PROBES TO STUDY BACTERIAL/ALGAL INTERACTIONS AND THEIR ROLE IN HARMFUL ALGAL BLOOM (HAB) ECOLOGY

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There is increasing evidence that bacterial-algal interactions play a role in Harmful Algal Bloom (HAB) ecology. Bacteria have been implicated in the production of paralytic shellfish poison (PSP) toxins, which are normally associated with bloom forming algal species, specifically toxic dinoflagellate algae. To clarify the role that these bacteria may play in the production of PSP toxins, it is desirable to identify and localise the toxic bacteria associated with the dinoflagellates and the toxic algal blooms that they produce. 16S rRNA-targeted probes offer the possibility for both and thus, probes have been made to putatively toxigenic bacteria isolated from the PSP-related dinoflagellate *Alexandrium tamarense* and tested for their specificity in dot blot and in-situ hybridisation experiments using cultured isolates. The bacteria belong primarily to the a-Protoebacterial group of Roseobacteria and the g-Proteobacterial group of *Alteromonas*. Results are also presented from field tests of the probes as well as localisation of these bacteria in cultures of the dinoflagellates using confocal microscopy. The major abundance of the bacteria recognised by the probes occurred at two sites around the Orkney Islands and preceded the major *Alexandrium tamarense* bloom, which also occurred at the same two sites, by two weeks.

COMPARATIVE RESPONSE TO FISH, ALGAL PREY, AND NUTRIENTS BY TOXIC, NONTOXIC, AND NEVER-TOXIC *PFIESTERIA PISCICIDA*

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We examined cell production in *P. piscicida* given fish versus algal prey. Clonal culture with demonstrated lethality to fish prey was isolated from the 1998 toxic *Pfiesteria* outbreak on the Neuse Estuary, North Carolina, USA., and was maintained in toxic (TOX, with live fish prey) and [temporarily] nontoxic modes (NONTOX, with cryptomonad prey) for 4 months prior to the experiments. A second clonal Neuse culture, isolated in 1997, was no longer toxic to fish and was grown on cryptomonads as a 'never-toxic,' kleptochloroplastidic strain (NEVTOX). Zoospores encysted when prey were allowed to deplete. Cysts were maintained for 1-26 weeks, with excystment triggered at initiation of experiments by adding fish or algal prey. The history of access to live fish strongly influenced subsequent cell production. When given fish prey, TOX zoospores had significantly higher rates of excystment and cell production than NONTOX and NEVTOX strains that previously had been grown with algae. When given algal prey (f/100 media), cell production by the NEVTOX strain generally was signficantly higher than by NONTOX (but potentially toxic) *P. piscicida*, with lowest cell production by the TOX strain that previously had been maintained in toxic fish-killing mode. Nontoxic zoospores became never-toxic – i.e., no longer showed attraction to fish or fish-killing ability – after 2-4 months on algal prey without access to fish. Under N or P enrichment with algal prey, highest and most rapid cell production was shown by the N-enriched NEVTOX strain, and a non-prey-mediated response was shown by the P-enriched TOX strain. The data from these experiments and similar research with other clones indicate that in culture, *P. piscicida* generally loses its fish-killing ability if maintained for more than short periods on algal prey; and that meaningful insights about the ecology of toxic *P. piscicida* cannot be obtained from research on never-toxic strains.

CYANOTOXIC BLOOMS IN FLORIDA'S (USA) LAKES, RIVERS AND TIDAL RIVER ESTUARIES: THE RECENT INVASION OF TOXIGENIC *CYLINDROSPERMOPSIS RACIBORSKII* AND CONSEQUENCES FOR FLORIDA'S DRINKING WATER SUPPLIES

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Cyanobacteria blooms are common in many of Florida's most important lakes, rivers and estuaries; threatening water quality, surface drinking water supplies, public health and aquatic ecosystems. In 1998, following the formation of the Florida Harmful Algal Bloom Task Force, a collaborative investigation of cyanotoxins in Florida?s surface waters was initiated by the St. Johns River Water Management District, Florida Marine Research Institute, Florida Department of Health and Wright State University. Project objectives include: (1) identify surface waters that experience extensive cyanobacteria blooms; (2) collect and identify potential toxigenic species; (3) screen samples for the detection and isolation of algal toxins; (4) characterize algal toxins present in water and animal tissues. Approximately 135 water samples have been collected from 125 surface water bodies throughout the state and analyzed for the presence of cyanotoxin(s); including microcystin(s), cylindrospermopsin, anatoxin-a, anatoxin-a(s) and paralytic shellfish poisons (PSPs). Methods used for the detection and characterization of algal toxins include mouse bioassay, enzyme-linked immunosorbent assay (ELISA), protein phosphatase inhibition assay (PPIA), anticholinesterase assay (AA), HPLC-MS/MS and HPLC-Fl. Results indicate that Anabaena sp., *Microcystis* sp. and *Cylindrospermopsis raciborskii* are the primary bloom forming cyanobacteria in Florida. The state-wide distribution of C. raciborskii and recent developments of severe blooms (>500 μg/L Chl a) and dominance (relative % biovolume = 97.4) by this species in many of Florida's lakes has raised concerns for ecological and human health. Surface water samples dominated by *C. raciborskii* were found to be toxic by mouse bioassay with HPLC results suggesting the presence of a compound similar, but not identical, to cylindrospermopsin identified from Australian waters. Relationships between surface water quality and the production of cyanotoxins, and concerns for present and future drinking water supplies, will be discussed in terms of specific management actions required to reduce the impact of harmful algal blooms in Florida.

THE EFFECT OF NITROGEN SOURCE ON THE GROWTH AND TOXICITY OF POTENTIALLY HARMFUL DINOFLAGELLATE SPECIES IN THE GENUS *PROROCENTRUM*

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Increases in population and agriculture in coastal areas can result in increased nutrient inputs and alterations in the ratios of organic to inorganic nutrients in coastal waters. Such changes in coastal nutrient regimes can affect phytoplankton community structure by creating conditions favorable for growth and dominance of algae that were not dominant before. The effect that changes in ratios and concentrations of nutrients have on toxicity of harmful algal species is not well known. There seems to be a relationship, however, between nutrient stress and toxin production among harmful phytoplankton producing low-N toxins, e.g. Diarrhetic Shellfish Poisoning (DSP) toxins. Even less is known about the relationship between organic nutrient uptake and toxin production. Benthic species and species in coastal areas are probably exposed to greater fluxes of dissolved organic nitrogen (DON). In this study, benthic and planktonic species of *Prorocentrum* were grown on L1 media with nitrate, ammonium, urea, L-Glutamic Acid, and natural DON as the sole N-source. DON from Mobile Bay was isolated and concentrated by tangential flow ultrafiltration for use in the natural DON treatment. Growth was measured throughout the complete growth cycle using in vivo fluorescence, flow cytometry, and direct cell counts. An ELISA specific to the DSP toxins, okadaic acid and 35-methylokadiac acid, was used to measure toxin concentrations. Preliminary results indicate that some organic forms of N support growth as well as inorganic forms, with urea actually supporting more growth than inorganic N-sources.

DETERMINATION OF BREVETOXINS FROM MANATEE TISSUES BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Reversed phase liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used to determine levels of brevetoxins (PbTX) in tissue samples from animals with suspected exposure to brevetoxins. With minimal cleanup, low levels (< pM concentrations) of individual brevetoxin congeners could be determined from a variety of tissue matrices. The utility of the approach is illustrated by application to tissue samples collected during a Manatee mortality event that occurred off of southwest Florida in 1996. Analysis of the tissue samples allowed unambiguous chemical determination of the presence of brevetoxin congeners in the tissues of animals exposed to a bloom of *Gymnodinium breve*. Typically, the levels of the brevetoxin congener PbTX-3 in blubber, liver, lung, and kidney tissues from intoxicated animals, as determined by the LC-MS/MS technique, were in the 0.1 nM^{-1} pM range. Furthermore, the specificity of the LC-MS/MS technique allowed the distinction between PbTX congeners and brevetoxin degradation products in the tissues. The degradation products, while having identical molecular weight and showing somewhat similar chromatography behavior to the brevetoxins, as well as exhibiting the ability to competitively bind to the brevetoxin receptor site in mammalian sodium channel preparations, were shown to be chemically distinct compounds by giving different fragmentation behavior in tandem mass spectrometry experiments.

LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY ANALYSIS FOR DOMOIC ACID IN SAMPLES ASSOCIATED WITH A SEA LION MORTALITY EVENT

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A part of our laboratory's response to the sea lion mortality event off of Monterey Bay, California (Summer 1998) was the quantitation of domoic acid (DA) in event related samples by a variety of techniques. For a definitive confirmation of the levels of DA, samples associated with the event were subjected to reversed phase liquid chromatography was coupled with tandem mass spectrometry (LC-MS/MS). With minimal cleanup, low levels (~10 nM concentrations) of DA could be determined from a variety of matrices. The LC-MS/MS analysis allowed unambiguous chemical determination of the presence of DA in various components of the food web in an area surrounding a bloom of the diatom *Pseudo-nitzschia.* Levels of DA in sea lion excrement from intoxicated animals and food web components, as determined by the LC-MS/MS technique were in the 1 μM-10 nM range. The specificity of the LC-MS/MS technique allowed the distinction between DA and coeluting components. These coeluting components, having a similar UV chromaphore and an identical molecular weight to DA, were shown to be chemically distinct compounds by giving different fragmentation behavior in tandem mass spectrometry experiments.

ALEXANDRIUM TOXICITY IN THE GULF OF TRIESTE (NORTHERN ADRIATIC SEA)

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In consequence of harmful algal blooms increasing everywhere, also in Adriatic Sea, many regional projects were promoted in the last decade to attend the ecology and oceanography of HAB in order to protect the human welfare. A monitoring program was carried out from August 1996 to November 1997 in the Gulf of Trieste to identify and study potentially toxic algae. Particular regard was devoted to the dinoflagellate genus *Alexandrium* such as its presence in the seawater as well as toxicity of cultures and blue mussels (*Mytilus galloprovincialis*) from shellfish farms in the Gulf. Chemical and physical parameters and water samples were collected monthly at four or five depths in four coastal stations in mussel farms. The biological data, based on light and electronic microscopy, revealed the occurence of three *Alexandrium* species and other 19 taxa of potentially toxic microalgae, but no harmful blooms occured in the studied period. The first presence of toxicity was found from a *Alexandrium* clone in which gonyautoxins were isolated too. The chemical analyses confirmed the presence of toxicity also in the mussels. The concentration of PSP toxins was always under the limit allowed by the Italian law, but the constant presence of PSP producers in the last years in the Northern Adriatic suggests that HAB and consequent human intoxications could increase in the future. There is still a lack of information regarding the temporal distribution and PSP population dynamics and further scientific investigation is required. An intensive check of the seawater and mussels quality is auspiced in order to safeguard the acquaculture activities and the public health.

ZOOPLANKTON GRAZING IMPACTS ON *ALEXANDRIUM* SP. IN THE GULF OF MAINE

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We investigated the grazing impact of dominant zooplankton on *Alexandrium* sp. populations during the spring of 1998 and 1999, in coastal waters of the Gulf of Maine. At weekly intervals, several stations were sampled for zooplankton and phytoplankton abundance, biomass, species composition, and toxin content. Using natural water samples and wild zooplankton collected from selected stations, we determined feeding rates of zooplankton in natural water samples containing *Alexandrium* sp. dinoflagellates. Dominant zooplankton included copepods (primarily *Acartia hudsonica* and *Calanus finmarchicus*) and barnacle nauplii (*Semibalanus* sp.). During 1998, *Alexandrium* sp. concentrations gradually increased during May, peaking at 3000 cells/L. One week after that peak, *Alexandrium* sp. concentrations fell to only 30 cells/L. Zooplankton community biomass was low during the initiation of the bloom, but increased exponentially during the period of our investigation. There was also a dramatic change in the zooplankton species composition during the bloom; barnacle nauplii dominated during the early phases of the bloom, and copepods, primarily *A. hudsonica*, dominated during the later phases. Grazing impacts were low during the bloom initiation and subsequent *Alexandrium* sp. increase. Concurrent with an increase in the *A. hudsonica* biomass, grazing impacts increased exponentially (peaking at about 70% / day) and appeared to contribute to the demise of the *Alexandrium* sp. bloom. In contrast, in 1999, concentrations of *Alexandrium* spp. remained very low throughout the study period. These findings suggest that grazing can be an important source of mortality and will depend on zooplankton clearance rates, degree of selective feeding, and the biomass and species composition of both the phytoplankton and zooplankton communities.

COMPARATIVE STUDIES ON MYCOSPORINE-LIKE AMINO ACIDS, PARALYTIC SHELLFISH TOXINS AND PIGMENT PROFILES OF THE TOXIC DINOFLAGELLATES *ALEXANDRIUM TAMARENSE, A. CATENELLA* AND *A. MINUTUM.*

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Surface bloom-forming species, predominantly of the Dinophyceae has the capacity of accumulate high amounts of MAAs. The three dinoflagellate species, *Alexandrium tamarense, Alexandrium catenella*, and *Alexandrium minutum*, are bloom-forming toxic isolates which by accumulating in surface waters are exposed to high light conditions. Using an improved HPLC methodology, nine MAAs were separated and identified and the presence of a series of atypical MAAs not previously reported in the literature was revealed. Their chromatographic behaviour, UV-spectra and chemical properties indicate that these compounds contain two or more common MAAs linked among them. Although some of these atypical MAAs are present in the three *Alexandrium* species, when comparing the chromatographic profile of A. minutum with those of *A. tamarense* and *A. catenella*, greater differences are seen. As the biochemical composition of cells is highly variable with growth conditions, we also report for a comparative discussion the toxin and pigment composition of these *Alexandrium* isolates. The three species showed the same pigment pattern characteristic of peridinin containing dinoflagellates. On the contrary, as reported previously, great variation of toxin profile was observed among the *Alexandrium* species. We conclude that, although MAAs are common among phytoplankton, the occurrence of novel MAAs in the three *Alexandrium* species indicate some degree of either biogeographic or ecotypic diversification.

POPULATION DYNAMICS AND SPIROLIDE COMPOSITION OF THE TOXIGENIC DINOFLAGELLATE *ALEXANDRIUM OSTENFELDII* IN COASTAL EMBAYMENTS OF NOVA SCOTIA

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Spirolides comprise a suite of pharmacologically active macrocyclic imines found in plankton size-fractions and shellfish from the eastern coast of Nova Scotia, Canada. A multi-year field program was initiated to establish the dynamics of these "fast acting toxins" and to determine the source organism. The successful culmination was the identification of the gonyaulacoid dinoflagellate *Alexandrium ostenfeldii* as the causative organism, and the isolation of a spirolide-producing clone. Analysis by liquid chromatography-mass spectrometry (LC-MS) showed that spirolide profiles were similar over time and depth within a site, but composition was markedly different among sites. For example, at Graves Shoal, NS, the primary components were B, D and its isomer D2, with A, C, and C2 as minor constituents, whereas at Ship Harbour, all size-fractions were particularly rich in a new spirolide, des-methyl-C, with lesser amounts of A and B derivatives. The spirolide profile at Graves Shoal has remained constant over several years, whereas the profile from Ship Harbour plankton showed inter-annual variation, suggesting a shift in dominance among different strains. The spatio-temporal distribution of spirolides in the water column is usually confined to late spring-early summer (May to July), following the decline in the spring diatom bloom. Physical data (temperature, salinity, (sigma-T) from the water column indicate that spirolide events are associated with a deepening of the pycnocline and the beginning of surface stratification. According to in vivo fluorescence and extracted chlorophyll a profiles, and data from long-term moorages of chain-sensors for up- and down-welling radiance ("ocean colour"), spirolide occurrence is not related to high plankton biomass. Highest spirolide concentrations are found in the 26-56 μm plankton size-fraction, during periods when large thecate dinoflagellates (e.g.,*Scrippsiella, Dinophysis, Gonyaulax, Alexandrium*) are dominant. Spirolide concentrations in the plankton at Graves Shoal, NS were highly correlated with the abundance of *Alexandrium* spp. ($r^2 = 0.93$), but attribution to *A. ostenfeldii* was complicated by the simultaneous presence of A. tamarense, a non-spirolide producing but morphologically similar species.

DEVELOPMENT OF A POTENTIAL HARMFUL ALGAL BLOOM IN A MESOCOSM EXPERIMENT MONITORED THROUGH PIGMENT ANALYSIS AND BIO-OPTICAL MEASUREMENTS

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As a part of MAST project NUTOX (Effect of nutrient ratios on harmful phytoplankton and their toxin production), we performed a mesocosm study at Trondhjem Biological Station, Norway, from 23rd August to 8th September 1999. Nutrient poor sea water was inoculated with natural phytoplankton, and exposed to possibly toxin-stimulating nutrient ratios. Seven different molar N:P ratios from 0.4 to 640 were studied, in addition to a control mesocosm where no nutrients were added. Physiological status of the cells was traced by means of bio-optical measurements (in vivo light absorption and fluorescence excitation spectra) and pigment analysis (HPLC), in addition to measurements of cell chemistry and toxin content. Exponential increase in chlorophyll a levels was observed shortly after the first nutrient additions. The development was parallel in all bags, but maximum levels of biomass were different. During this period the phytoplankton assemblages were dominated by haptophytes, which can be traced through pigment composition (presence of chl *c* s and 19,hexanoyloxy- and 19,butanoyloxyfucoxanthins) and their characteristic absorption properties. [Chl a] levels in all bags decreased rapidly after reaching the first maximum, and then we observed a second increase in [Chl a] but this time the biomass increased in a less clear pattern. Grazing by ciliates seemed to make an impact on the phytoplankton assemblages, and a change in dominating groups was also observed: Small prasinophytes and a variety of dinoflagellates were found at this stage. We observed a change in pigment contents as well, when chlorophyll b became more prominent at this later stage of the experiment. In this work the relationship between different phytoplankton assemblages, pigmentation and bio-optical characteristics is discussed.

WHAT SHOULD WE DO AFTER THE SEVERE 1998 HAB EVENT IN HONG KONG?

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A severe harmful Algae Bloom (HAB) hit Hong Kong and its adjacent areas in 1998, causing more than 300M HK$ lost and social chaos. The main concern was: will the event happen again? When? Several steps have been taken towards this question" What should we do after the event". HK government has organized an expert committee to study necessity and possibility to set up a program of "red tide monitoring and management in Hong Kong". And the AFD(Agriculture and fisheries Department) and EPD(Environmental Protection Department) in Hong Kong has strengthened routine monitoring activities around HK waters. The academic community in HK also actively participated to answer the question. Three projects, sponsored by different source, every one focused on HAB related issues, were integrated and launched investigations in 1999. The subjects in the integrated projects were 1. Dynamics of nutrients in HK and adjacent seas. 2. Mechanism of eutrophication and its relation to HAB in HK. 3. Understanding of limiting/promoting factors to local HAB species. 4. Competition of "native" species with invaded species in forming HAB in this area. 5. Development of 3-D monitoring system and early warning model for HAB alert/ alarm signal. Preliminary results showed that 1. It is difficult to solely use satellite images for HAB study in this area because of the weather (>90% clouded), additional data processing may help t o improve the output. 2. Some kind of correlation seems to existed between El-nino and HAB events in this area. 3. Potential for two local species to form HAB in this area were found after series of environmental condition experiment. 4. It revealed that meteorological conditions may pay an important role in providing nutrients in forming HAB if the wind direction is "correct" and strong enough to remove the surface water in a specific area to form "small scale upwelling".

MOLECULAR CHARACTERIZATION AND CLASSIFICATION OF THE CIGUATERA DINOFLAGELLATE *GAMBIERDISCUS*

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The benthic dinoflagellate *Gambierdiscus* is regarded as the primary causative agent of Ciguatera Fish Poisoning (CFP), a disease prevalent in the Pacific regions, the Carribean and the Indian Ocean. Six distinct species within this genus, most of them being toxic, have been described so far on the basis of morphological criteria. The genetic characterization of various strains of *G. toxicus* has also been addressed in recent studies. In the present work, we investigated the usefulness of the rRNA genes for the molecular characterization and classification of 15 clones of Gambierdiscus distributed in 5 of the 6 species described to date. The 5.8S+ITS and the LSU rDNA D8-D10 regions of 11 Polynesian isolates were PCR-amplified prior to their cloning and sequencing, for phylogenetic analysis by means of sequence comparison. Both regions proved to be useful biogeographical markers, as a grouping of these isolates according to their geographic origin was globally observed. To investigate the potential interest of the LSU rDNA D8-D10 regions in Gambierdiscus systematics, sequences of 8 isolates from distinct geographic origins, distributed among the *morphospecies G. toxicus, G. yasumotoi, G. polynesiensis, G. australes* and *G. pacificus*, were also compared. Four molecular types could be distinguished, which tend to indicate that species designations in this genus based on SEM microscopy are consistent with classification inferred from genetic heterogeneities. The relevance of a modern form of taxonomy in dinoflagellates that would combine both molecular and traditional morphological criteria, is also discussed.

THE APPLICATION OF SPECIES-SPECIFIC DNA-BASED PROBES AND FLUORESCENT TAGGED LECTINS FOR DIFFERENTIATION TOXIC *PSEUDO-NITZSCHIA MULTISERIES* FROM NON-TOXIC *P. PUNGENS*

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It has been known that several species of *Pseudo-nitzschia* produce a toxin, domoic acid (DA), responsible for the syndrome commonly referred to as amnestic shellfish poisoning (ASP), and then make a serious impact on shellfish farm in several countries. There have been no reports on risk assessments of DA contamination of shellfish in Korea. However, recently, we have observed potentially toxic *P. multiseries* and non-toxic *P. pungens* using scanning electron microscope, which are very similar morphologically and are considerable confusion under the photomicroscope. It is therefore desirable to detect and enumerate such species for harmful algae monitoring and prediction systems. In this study, we applied DNA probes and fluorescent tagged lectins to differentiate toxic *P. multiseries* from non-toxic *P. pungens* isolated from Chinhae Bay. From the binding activity of lectins, two species were responsible for bright fluorescence on the cell surface of ConA and RCA, whereas PNA, UEA and DBA presented no fluorescence with P. multiseries and *P. pungens*. In particular, fluorescent WGA specifically bound with *P. multiseries* but not with *P. pungens*, indicating a desirable method of rapid and easy discrimination between tested *Pseudo-nitzschia* species. In addition, we have tested species-specific oligonucleotide DNA probes to these two species with the aid of whole cell hybridization by filter tube system. The positive control (uniC) probe bound to both *Pseudo-nitzschia* species, but negative control (uniR) and *Alexandrium tamarense* (NA1) probes did not bind to both species. However, *P. multiseries* DNA probe (muD1) was negative for *P. pungens* under the epifluorescent microscope, suggesting that these organisms were recognized by their own specific oligonucleotide probes and distinguished between positive and negative control probe. Thus this technique is suitable to differentiate toxic *P. multiseries* from non-toxic *P. pungens* for a specific fragment of rRNA.

HARMFUL ALGAL BLOOM AND PRIMARY PRODUCTION IN ARTIFICIAL LAKE SHIHWA

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Lake Shihwa, which was artificially formed by the construction of dike on the intertidal flat in 1994, represent perennial eutrophication and hypoxic condition in the water columns after construction. The dense phytoplankton blooms of average value of 168 μgchl-a l-1 have occurred. This eutrophication is resulted from the large input of nutrients from the industrial complex and populated cities in the hinterlands. The major organisms of algal blooms were the dinoflagellate, *Prorocentrumminimum*, in spring and summer and diatoms in autumn and winter. The autumn and winter diatom blooms were limited by the depletion of silicate in the lake. The primary productivities in the lake Shihwa ranged from 2650mg Cm2 day to 9500mg Cm2 day with an average of 3970mg Cm2 day. These high primary productions were limited to the shallow euphotic zoned use to the inhibition of light by high algal biomass on the surface layer. Lack of photosynthesis and the decomposition of falling organic matter under the middle of water column accelerated the depletion of dissolved oxygen in the bottom layer. The lake of mixing within the water column was also one factor of anoxic condition in the deepest portion of the lake.

SPIRO-PROROCENTRIMINE, A NOVEL MACROCYCLIC LACTONE FROM BENTHIC *PROROCENTRUM* SP.

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A polar lipid-soluble toxin, spiro-prorocentrimine has been isolated and crystallized in a separation of prorocentrolide from an unknown benthic *Prorocentrum* species. This compound, C42H69NSO13, has similar macrocyclic structures as prorocentrolide, a toxin from a strain of *P. lima,* but a smaller macrolide moiety. Like the toxin, prorocentrolide B, *from P. maculosum* spiro-prorocentrimine contains a sulfonyl substitution on the 6-membered ether ring instead of the 5-membered cyclic ether within the macrolide moiety of prorocentrolide B. The unique feature of the compound is the spiro-linked cyclic imine with the ortho, para-disubstituted 3 'cyclohexene, a characteristic functional group also found in gymnodimine, rather than the hexahydroisoquinoline moiety on both prorocentrolide and prorocentrolide B. Structure of this compound was established by NMR spectroscopy and x-ray crystallography. This is the first compound of this group of marine toxins with a clear stereochemistry.

EFFECTS OF IRON AND MANGANESE CONCENTRATION AND THEIR RATIO ON CELL GROWTH AND TOXIN PRODUCTION OF THE CYANOBACTERIUM *CYLINDROSPERMOPSIS RACIBORSKII*

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The control of toxic cyanobacterial blooms is of great importance to public and environmental health. Little is known about the nutrient requirements and the relationship between nutrient availability and toxin production in *Cylindrospermopsis raciborskii,* one of the common potentially toxic cyanobacteria found in Queensland. Manganese (Mn) and iron (Fe) are essential nutrients at low levels but potentially toxic at high levels. Further, despite the fact that Mn and Fe have been found at elevated levels in waterbodies in Queensland, little is known about the tolerance of *C. raciborskii* to limited or elevated levels of Fe and Mn. The objective of this study was, to identify the potential effects of varying Mn and Fe concentrations and their ratios on cell growth and toxin production in *C. raciborskii* grown in batch cultures.

Manganese was neither limiting nor inhibiting to growth and toxin production of *C raciborskii* at initial Mn concentration from below the detection limit of 2 μg L-1 to 2.3 mg L-1. Furthermore, in the study of manganese toxicity in relation to iron availability in the medium, Mn toxicity could not be induced at initial Mn concentrations up to about 1.5 mg L-1. However, iron induced growth limitation was observed at iron concentrations of about 10μg L-1 or less. The results further suggest that a linear relationship with a slope of unity between the growth rate constant (c) and the toxin production rate constant (CYN) existed when Fe was limiting. This finding suggests that toxin production was indirectly controlled by the effect of Fe limitation on the rate of cell division and not through any direct effect on metabolic pathways of toxin production. Thus the authors propose that the toxin, cylindrospermopsin is a secondary metabolite, but not a siderophore.

THE FIRST WIDESPREAD OUTBREAK OF *GYMNODINIUM SP.* IN SOUTHERN CHILE.

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A massive outbreak of phytoplankton was observed in southern Chile during March and April of 1999. The predominate phytoplanktonic species, more than 99%, was *Gymnodinium* sp.. This species was presented in dense red patches and the highest cell concentration was 8-9 million cell/L. This event caused a high mortality in shellfish (clam, sea urchins, abalone), polychaete, and fish (farmed salmon and wild species), the hardest hit being the farmed salmon.

Several factors suggest that this bloom originated in the open sea. One possible cause of the event could be the current climate anomaly during the last 14 months in the region, with drought conditions and strong sunshine. These factors produced a 1.5 C increase in the surface water temperature, in the surrounding area (15 C). At the time of the bloom, the toxicity and the allelopathic properties were studied in the contaminated seawater under laboratory conditions. The toxicity was evaluated using a haemolytic test and the allelopathic activity was studied on two species, a diatom (*Leptocyndrus minimus*) and a dinoflagellate (*Alexandrium catenella*). Both of these produce red tides in southern Chile, and the *Gymnodinium* sp. density in the allelopathic experiment was 800 cell/ml. The haemolytic activity was very high with *Gymnodinium* sp. concentration of 4.000 cell/ml. A diluted sample (250 cell/ml) still produced significant activity, with 18% haemolysis. In the allelopathic experiments, the growth of the diatoms was totally inhibited in water containing *Gymnodinium* sp, while the growth of *A. catenella* was practically not affected. When this seawater was passed through Sep-Pak and Florisil cartridges, the inhibitory effect on the diatom growth was suppressed. This behavior is similar to other ichthyotoxic species of *Gymnodinium* present in other parts of the world. The biological properties of this species, along with the punctual climatic and hydrological conditions, facilitated the development of this bloom which had the largest geographical coverage ever recorded in Chile.

DINOFLAGELLATE PARASITISM: INFLUENCE OF NUTRIENT ENVIRONMENT ON PARASITE SUCCESS AND EFFECT OF INFECTION ON HOST SWIMMING SPEED AND PHOTOSYNTHESIS

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Preliminary attempts to culture *Amoebophrya* sp. ex. *Gymnodinium sanguineum* from Chesapeake Bay indicated that parasite success may be influenced by water quality. To explore that possibility, we determined parasite generation time, reproductive output, and survivorship of progeny (i.e., dinospores) for host-parasite populations grown in high, intermediate, and low nutrient medium. Parasite generation time normalized to host cell volume showed significant differences among treatments, with infections progressing most rapidly in nutrient replete medium. Parasites of hosts grown in nutrient replete medium also produced 3-4 times more dinospores that those infecting host under low nutrient conditions. Furthermore, the percent of dinospores that established new infections was significantly greater for "high-nutrient" vs. "low-nutrient" parasites. In separate experiments, we determined swimming speed and photosynthetic performance of synchronously infected *G. sanguineum* relative to parasite age. Host swimming speed decreased gradually throughout the infection cycle, showing a 40% total reduction prior to death of the host. By contrast, photosynthesis at saturating irradiance decrease abruptly by about 60% early in the infection cycle and then remained stable through maturation of the parasite. Results indicate that *Amoebophrya* sp. is well adapted to exploit host populations of enriched coastal environments and exerts significant controls on host behavior and physiology.

THE INVESTIGATION OF A DINOFLAGELLATE ASSOCIATED WITH A FISH KILL EVENT IN THE MURRAY RIVER/ESTUARY, WESTERN AUSTRALIA.

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Two brief fishkill episodes occurred in the Murray river, Western Australia, on June 2 and June 8 1999. Physical profiles of the water column were taken to check for deoxygenation, while integrated and grab phytoplankton samples were also collected for analysis. With only low levels of H2S and NH3, and satisfactory O2- concentrations, none of the physical attributes of the water column could be linked to the fishkill. Pathological tests on fresh dying fish collected from the site concluded that liver and gill damage symptoms were consistent with effects of toxic dinoflagellate algae. Substantial concentrations of a small dinoflagellate (12-17μm) were detected close to the reported fishkill site on June 2 and again on June 8. With normal light microscopy techniques this dinoflagellate was tentatively identified as *Gymnodinium cf. galatheanum* Braarud. The identity of the dinoflagellate in question was further investigated with techniques including Nomarski (DIC) phase microscopy, fluorescence microscopy and electron microscopy. Evidence gathered suggests that the dinoflagellate species observed in the Murray River at the time of the fishkills was *Gymnodinium galatheanum* Braarud, a species previously reported to be icthyotoxic (Abboud-Abi Saab, M. & Y. El-Bakht, 1998; Johnsen, G. et al., 1998).

PSEUDO-NITZSCHIA SPP. IN IRISH COASTAL WATERS

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A sample set generated from field investigations of phytoplankton in Irish coastal and shelf waters carried out since 1993, was used to determine the temporal and spatial variability of species within the genus *Pseudo-nitzschia*. The study was carried out to ascertain the threat posed by this diatom to the Irish aquaculture industry. Six *Pseudo-nitzschia* spp. were positively confirmed using electron microscopy *(P. pungens, P. multiseries, P. fraudulenta, P. australis, P. delicatissima* and *P. pseudodelicatissima*). *Pseudo-nitzschia seriata f. seriata, P. seriata f. obtusa*, and*P. subpacifica* were tentatively identified and a further unidentified *Pseudo-nitzschia* species was also observed. One species (*P. australis*) isolated from Irish waters produced domoic acid (DA) under laboratory conditions. A further six of the species present have been reported as DA producers in the literature. There was no apparent relationship between species dominance with either season or hydrography. *Pseudo-nitzschia pungens* (June 1997 and August 1993) *P. pseudodelicatissima* (July, 1996) *P. australis* (September 1996), *P. delicatissima* (May 1996 and September 1996) and *P. fraudulenta* (October 1997) were at varying times dominant, either within the genus or the total phytoplankton population, in cell concentrations of up to 2 000 000 cells per litre.

SHELLFISH TOXICITY IN NORWAY – EXPERIENCES FROM REGULAR MONITORING, 1992-1999

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Shellfish toxicity and the presence of toxin producing phytoplankton have been monitored along the Norwegian coast since 1992 in nearly the same way concerning frequency (weekly) and methods for sampling, microscopy and toxin extraction and analysis. The number of sampling sites has increased from 17 in 1992 to 24-27 after 1994, now covering the whole Norwegian coastline between Sweden and Russia. The cumulated information gives a comprehensive knowledge of the variations in shellfish toxicity and the occurrence of toxic species along the Norwegian coast and is a consistent basis for intercomparisons between regions and years.
The combined phytoplankton and toxicity data demonstrate that DSP and PSP may occur all along the coast from 58°N to 70°N, with large interannual and regional variations. In general the risk of DSP is highest along the south coast and in the inner parts of the large fjords along the west coast in the autumn/winter period (September-February). PSP occurs all along the coasts, with some hot spots at the northwest coast (62-64°N). The risk is highest in late spring and early summer (April-July) in southern Norway and a couple of months later in northern Norway. Although several blooms of potentially ASP producing diatoms have been observed, domoic acid /ASP has so far not been documented in bivalves from the Norwegian coast.
Every year all sampling sites, with few exceptions, experienced periods with toxic shellfish, and at the same time up to 15-16 of 27 stations were closed for shellfish harvesting due to the risk of intoxications.During the period 1992-99 the northern limit of documented presence of DSP in mussels was moved from Vega (66°N, 13°E) to Alta (70°N,23°E) in 1999 and PSP from Trondheim (63°N, 12°E) before 1992 to Vadsø (70°N, 30°) in 1997. The observation period is probably too short to conclude concerning trends of spreading in toxic phytoplankton in this region. Our data do not support a clear linkage between eutrophication and the risk for PSP or DSP.

RELATIONSHIP BETWEEN *DINOPHYSIS* SPECIES AND SHELLFISH TOXICITY

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The three most common *Dinophysis* species along the Norwegian coast, *Dinophysis acuminata, D. acuta* and *D. norvegica*, can all contain Diarrhetic Shellfish Toxins (DST). The concentrations of these algae have been counted 3 times a week since 1989 on the south coast of Norway. The relationship between *Dinophysis* concentrations and DST in blue mussel (*Mytilus edulis*), as measured by mouse bioassay approximately fortnightly between 1994 and 1998, was studied by Pearson correlation analysis. No significant relationship was found between DST and cell concentration of neither species. However, after weighting the concentrations of algae by the inverse chlorophyll a (Chl a) concentrations (*Dinophysis* contribute generally very little to total Chl a), a positive correlation was found between DST and the occurrence of *D. acuta,* but not between DST and the other *Dinophysis* species. Hence, there seems to be complex relationships between the occurrence of *Dinophysis* spp. and DST in blue mussels. Possible explanations may be: 1) high variability of the toxin content per cell of *Dinophysis*, 2) dampening effect of non-toxic phytoplankton in the diet of shellfish, 3) existence of discrimination and/or selection mechanisms for phytoplankton in the shellfish, 4) shifts in shellfish filtration rates and/or depuration rates.

TOXIN ANALYSIS IN THE CYANOBACTERIUM *NODULARIA SPUMIGENA* – A COMPARISON OF ANALYTICAL TECHNIQUES

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The cyanobacterium *Nodularia spumigena* is omnipresent in the Baltic Sea, but can also be found in brackish waters world-wide. It produces nodularin, a pentacyclic peptide which exhibits hepatotoxic activity. Due to the occurrence of *N. spumigena* in drinking water reservoirs specific, straightforward, and rapid procedures are required for the detection, identification and quantification of the toxin.

The fact that nodularin produces its toxic effects through the specific inhibition of the cell regulatory enzymes serine/threonine protein phosphatases PP1 and PP2A allows to employ protein-phosphatase-inhibition assays. Results of these are compared to those obtained by a bioluminescence assay and with high performance liquid chromatography with both UV and mass spectrometric detection.

Aspects of specificity, quality, efficiency, detection limits and applicability of the different methods are discussed. A flow scheme for analyses of cyanobacterial samples from screening to exact analysis is suggested.

TROPHIC EFFECTS OF ESTUARINE BLOOM SPECIES ON BENTHIC AND PLANKTONIC GRAZERS

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Harmful algal blooms (HAB) may result from higher growth rates, lower mortality, physical aggregation, or some combination of these factors. We have been focusing on grazing mortality as a mechanism that may be involved in both bloom initiation and demise. In laboratory experiments, we are examining the effects of several estuarine HAB species on common benthic and planktonic grazers, including larval and juvenile bay scallops (*Argopecten irradians*), the copepod *Acartia tonsa*, and a variety of ciliate microzooplankton. We are measuring feeding and growth rates and examining sublethal effects using histological methods. To date, most of our efforts have been concentrated on the dinoflagellate Prorocentrum minimum (strain EXUV) and the raphidophyte *Heterosigma carterae* (strain OL). In temperate estuaries of the US Atlantic coast, these species frequently bloom in summer, and blooms appear to originate in bays and subestuaries, subsequently proliferating into open estuarine waters. Our preliminary results are consistent with the idea that inability of benthic grazers to control these species in shallow nearshore waters results in high populations that are exported to deeper waters of the estuary, where planktonic grazers gradually reduce their numbers. *P. minimum*, for example, is readily eaten by variety of ciliates, as well as the copepod, but rejected in pseudofeces by the scallop. In addition, this HAB species causes pathological changes in the digestive systems of juvenile scallops when it is ingested.

ALGAL BLOOMS MONITORING WITH A TELEMETRIC TECHNOLOGY : SEAWATCH INDONESIA BUOY

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Field sampling is a usual method for monitoring the algal blooms in Indonesia. As a new alternative for monitoring the algal blooms, SEAWATCH Indonesia project has been assesst the OPTISENS (an optical sensor) for monitoring the algal blooms phenomena in some SEAWATCH buoy sites (Jakarta Bay and Jepara). The coefficient attenuation data produced from the OPTISENS in a near real time manner and transfer via satellite to the read down station at SEAWATCH Indonesia office at BPPT building. Principally the OPTISENS was design to determine particles (phytoplankton or others) at the wave length 650, 555 and 470 nm. By processing the data (until get the ratio of coefficient attenuation relative) and combine with field sampling and others environmental data (oxygen saturation, salinity, brightness, etc.), this SEAWATCH system can be used for monitoring the algal blooms as an early warning system. The result from coefficient attenuation data processing from Jepara buoy shown a high abundance of phytoplankton at that area and the result from field sampling shown that at those time Jepara waters around the buoy site was dominated by *Nitzschia, Chaetoceros* and *Rhizosolenia.* But, the result from Pluit buoy coefficient attenuation data processing didn't show the domination of phytoplankton. The composition of the ratio : c'(B)/c'(R) <1; c'(B)/c'(G) <1 and c'(G)/c'(R) >1 shown others particle dominated Pluit waters at those time.

TOXIN ANALYSIS OF *ALEXANDRIUM TAMARENSE* STRAINS FROM SOUTH BRAZIL

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The first records of *Alexandrium tamarense* in the South Atlantic Ocean were reported in 1980 in Uruguay and Argentina, while in southern Brazil, the species was first discovered in 1996 when cell densities reached 2x105 cell/L near the Lagoa dos Patos estuary inlet. 4 strains of *Alexandrium* were isolated from this area, and their toxins were analyzed using post-column derivatization HPLC methods. All 4 cultures showed a similar toxin profile with high proportions (>44% on a molar basis) of the toxins C1,2 and lesser amounts of GTX1,4, GTX2,3 and NEO. Toxin content values ranged from 8,396 to 53,063 fgSTXcell-1, the latter value on par with the most toxic isolate from northeastern North America that has been analyzed by HPLC. While there is no shellfish toxicity data available from this region, the high toxicity of this one isolate, coupled with the dense concentration of *Alexandrium* observed in Brazilian coastal waters, suggest that there is potential for significant levels of PSP toxins to accumulate in shellfish. This could pose a serious risk to consumers, as is also the case along the Chilean and Argentine coasts. In addition to the toxin data, information on cellular growth rate as a function of temperature, light and salinity will be presented, and restriction fragment-length polymorphism (RFLP) data for these 4 isolates will be reviewed to suggest how they are phylogenetically related to other *Alexandrium* isolates on a global scale.

THE EFFECT OF PHOSPHORUS SUPPLY ON GROWTH AND TOXIN PRODUCTION OF *CYLINDROSPERMOPSIS RACIBORSKII*

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Cylindrospermopsis raciborskii, a cyanobacterium commonly isolated from tropical to subtropical freshwater sources has the potential to produce a potent hepatotoxin, cylindrospermopsin. The presence of this organism and its toxin is of major concern to Queensland water management authorities, as its toxin has been associated with human health problems and livestock deaths. It is well accepted that phosphorus is an important element in the formation of cyanobacterial blooms and that it may play a role in regulating the toxicity of some cyanobacterial species. However to date neither the phosphorous requirements *of C. raciborskii* nor the effect of phosphorus on the growth rate and toxin production of *C. raciborskii* has been studied in detail. The aim of this project was to investigate the effect of various phosphorus concentrations on the growth rate and toxin production of *C. raciborskii.*

The study was undertaken in two experiments in each of which *C. raciborskii* was grown in batch culture for approximately a duration of 30 days under continuous illumination, with varying initial phosphorus concentrations being supplied to the different test cultures. The results obtained showed that phosphorus concentrations as low as 10 μg L-1 were adequate for significant growth to occur and that concentrations below 5 μg L-1 were limiting to cell growth. Toxin production was shown to be related to cell concentration and no significant proportional change in toxin concentration with respect to cell count occurred due to changing phosphorus concentrations. The outcome of this study suggests that phosphorus is not an environmental trigger for toxin production in *C. raciborskii*, but may affect the growth and cell concentration leading to increased toxin concentration in the water.

THIN LAYERS AND HARMFUL ALGAL BLOOMS: AN EPHEMERAL PHENOMENON, OR A RECURRENT PATTERN?

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Many of the organisms which form harmful algal blooms are known to occur in thin layers. In some cases, layer formation is due to behavior of the organism. Alternatively, it may result from biological-physical interactions. Recent innovations in optical/physical instrumentation and techniques for collection of high-resolution data have greatly improved our ability to define the temporal and spatial extent of layers, and to investigate the role of biological-physical interactions in controlling their dynamics. This may be critical to the understanding of some kinds of HABs, because the dynamics and impacts of a population concentrated into a thin layer may be very different from that of one spread throughout the water column.

The first step to predicting the occurrence of thin layers is documentation of how often, and under what circumstances they occur. As part of an effort to develop and field test instruments designed to quantify thin layers, we collected a data base of more than 150 physical-optical profiles over a three month period in the summer of 1996 in East Sound, Washington, USA. Thin layers are a recurrent phenomenon in this fjord, and they can be dominated by, or contain harmful algae. Thin layers occurred in over 54% of our profiles. Their depth and intensity was closely associated with the depth and strength of the pycnocline. Over 72% were located at the base of, or, above and in the pycnocline. Of the remaining layers sampled, roughly 14% were associated with water masses being advected into the system below the primary pycnocline, while the final 14% occurred when the pycnocline was diffuse. More than half were found in regions of significant current shear. When the horizontal and vertical patterns of layer distribution are coupled with measurements of currents, it becomes clear that the patterns are strongly influenced by basin-scale circulation.

ALGAL BLOOMS IN FRENCH POLYNESIAN ATOLLS, TOXIC OR ANOXIC?

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Known since 1907, algal blooms remain poorly documented in French Polynesian atoll lagoons. Planktonic blooms often occurred in unhabited places and had a short duration. Researches were preferentially focused on the benthic dinoflagellate *Gambierdiscus toxicus*.

The development of pearl oysters mariculture, now the main economic resource, drastically changes this point of view, especially since 1994 when a bloom devastated the whole benthic fauna of Hikueru Atoll, including pearl oysters and fishes. As samples, obtained only one month later, showed in abundance a small coccoid chlorophyte, it was not possible to attribute the mortality either to a toxic algae or to anoxic environmental conditions. In 1997, another bloom caused severe damages to reared pearl oysters in Manihi Atoll. Samples taken during the bloom only revealed the presence of a small unknown holococcolithophorid, i.e. a family which has never been recorded as toxic. Given the localized occurence of the bloom, anoxic conditions seemed to be very unlikely and the causes of the animal death remained undetermined.

The causes of mortality induced by algal blooms however have to be determined in order to prevent their consequences. If toxic species are involved, it can lead to human diseases and the seafood consumption should be temporarily prohibited. If algal blooms cause anoxic conditions, special settings, such as the displacement of reared pearl-oyters, must be forecasted. In order to evaluate the possible occurence of toxic species, taxonomic surveys of the small phytoflagellates are conducted in various atolls in order to list the species which are suspected to bloom. First results confirmed the supposed threat as the often toxic genus *Chrysochromulina* was found in all the atolls surveyed.Further planned researches include the complement of the taxonomic list of phytoflagellates as well as the determination of their possible toxic character using laboratory cultures.

NUCLEAR PROTEINS POTENTIALLY INVOLVED IN THE CONTROL OF THE TRANSCRIPTION IN THE HETEROTROPHIC DINOFLAGELLATE NUCLEAR PROTEINS POTENTIALLY INVOLVED IN THE CONTROL OF THE TRANSCRIPTION IN THE HETEROTROPHIC DINOFLAGELLATE *CRYPTHECODINIUM COHNII.*

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Dinoflagellates are biflagellated unicellular eukaryotes widely spread all over the aquatic world and well-known for their harmful blooms. They are characterized by their chromosomes always condensed during the cell cycle and by an unusual mitosis called dinomitosis which shows an extranuclear spindle crossing the permanent nuclear envelope via cytoplasmic channels. One of their prominent features which is unique among the eukaryotes is the absence of histones and nucleosomal organization. This raises the problem of condensation of a high quantity of their genomic DNA as well as the replication and transcription mechanisms. Dinoflagellates could use some divergent alternative mechanisms for these prosesses probably involving new proteins. We already described a nuclear protein Dinap1 (Dinoflagellate nuclear associated protein 1) expressed only in G1 phase, in the species *Crypthecodinium cohnii*. The presence of two WW domains led to the hypothesis that Dinap1 could form a multiprotein complex involved in the transcription regulation. WW domains are known as protein-protein interaction modules which recognize a proline-rich sequence. We used the protein-protein recognition property of the WW domains to screen a cDNA library of *C. cohnii* and find Dinap1 ligands. Five differents clones called Dip for Dinap1 interacting protein have been isolated. The Dip1 protein contains a PPTY motif, able to bind to the Dinap1 WW domains, and displays two new WW domains. To address the question of the Dinap1-Dip1 complex function, a new screen was performed to identify Dip1 ligands. This led us to the isolation of three new proteins, Dap B, Dap C and Dap G for Dip1 associated protein. The interactions between all these proteins as well as their abilities to bind DNA and their action in an in vitro transcription assay have been investigated.

MONITORING AND MANAGEMENT OF HARMFUL ALGAL BLOOM EVENT ON THE BLACK SEA COAST OF GEORGIA

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In the eastern part of the Black Sea harmful algal bloom problem is exists in the Paliastomi lagoon. Till 1934 Paliastomi was known as a fresh-water lake in the Black Sea coastal zone; than it was artificially connected to the Black Sea and is now a lagoon. Studying of this problem has begun since 1964. The identification of phytoplankton in previous years showed that blooms were caused by excessive growth of brackish-water toxic species Nodularia spumigena f.litorea, *Anabaenopsis elenkinii* and *Protoperidinium globulus* var. ovatum, and non- *toxic Rhizosolenia fragilissima* and *Anabaenopsis arnoldii*. These events repeated periodically in August-September and were associated with fish kills. Researches done by us in 1998-99 showed obvious changes of phytoplankton species in Paliastomi lagoon. The samples collected in 1998 (August- September) revealed the presence 76 species and samples collected in 1999 (September-August) revealed the presence 55 species, among them were: Cyanophyta, Bacillariophyta, Pyrrophyta, Xanthophyta, Euglenophyta , Chlorophyta, in all 55 taxons. The species identified include *Microcystis aeruginoza* Kutz, *Anabaena flos-aqua* (Lyngb.), *Oscilatoria* sp., *Cyclotella kuertzingiana, Coscinodiscus lacustris* Grun. Most of phytoplankton species were fresh-water forms unlike from previous years. It has become clear that brackish-water and marine species has changed to fresh-water species. It may be caused by frequent lock of the channel in Paliastomi during the recent two years and by lessen of marine water from the Black Sea. Paliastomi is located in conservation of Kolkhi Lowland and is under the integrative management. The program of Integrative Management of the Black Sea Coastal Zone includes assessment of risk posed by harmful algal blooms in the Black Sea coastal environment and restoration the stock of fishes (during the last decade species of fishes reduced from 39 to 10) and other living resources in this area. It is considered that one of the reasons of eutrophication and harmful algal bloom events in Paliastomi is flowing of contaminated waters. Monitoring of harmful algal bloom is going for restoration the biodiversity in Paliastomi lagoon.

THE CYANOBACTERIA *APHANIZOMENON FLOS-AQUAE* ISOLATED FROM PORTUGUESE FRESHWATER: OBSERVATIONS ON DEVELOPMENT, MORPHOLOGY AND TOXICITY DURING GROWTH PHASES.

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Aphanizomenon flos-aquae was isolated in 1996 from a toxic bloom at Montargil reservoir, Portugal. This strain has been successfully cultured with sustained paralytic shellfish poison (PSP) production in Z8 medium, at 20+or-1 oC with constant air supply, using a 16/8 hours L/D cycle by fluorescent lighting. Under this conditions, growth rate was determined, changes in trichome and cell morphology were examined, and cell-associated and media-associated toxins were analysed during initial, exponential and stationary growth phases. Cell-associated PSP toxins were analysed from the cultured cells concentrated either by decantation or by gentle centrifugation and extracted twice with 0.1 M chloridric acid and methanol:chloroform (1:1) solution. Media-associated PSP toxins were quantified from algae-free filtrates from each culture, acidified to pH 4 and concentrated in a rotary evaporator. Both cell-associated and media-associated toxin contents were analysed by HPLC-FLD postcolumn derivatization method, under different postcolumn derivatization conditions. Toxin profile was constant throughout the culture growth. Toxins production per cell increased from $1,9 \times 10^{-2}$ fentomoles of saxitoxin analogues per cell at the initial growth phase to $11,2 \times 10^{-2}$ fentomoles per cell at the late stationary ($14,9 \times 10^{6}$ cell/millilitre). Toxin content in the free-cell medium also increased with culture growth, reaching 250 fentomoles of saxitoxin analogues per millilitre at the late stationary phase. These data indicate that maximum rate of PSP production and concentration, both inside the cells and within free-cell media is reached at the end of the growth cycle.

USING OF THE SATELLITE INFORMATION FOR ANALYSIS OF THE BALTIC SEA PHYTOPLANKTON COMMUNITY

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The SeaWiFS satellite data about the radiation from the Baltic Sea surface were compared with synchronous instrumental measurements of the phytoplankton biomass and abundance in the surface layer of the sea. The correlations were estimated between the phytoplankton biomass in the surface layer and intensity of different spectral bands of the SeaWiFS water-leaving radiances. The best correlation coefficient was 0.85. Using these statistics and the developed algorithm the field of biomass for whole Baltic Sea was reconstructed. Calculations of the chlorophyll a concentrations were performed also. The results were compared with standard SeaWiFS level 2 product. Attempt to determine phytoplankton species composition was made.

A CALCIUM DEPENDENT ALLELOPATHIC EFFECT OF THE DINOFLAGELLATE *COOLIA MONOTIS* ON THE CHLOROPHYCEAE *DUNALIELLA SALINA*

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A new allelopathic effect of the ciguatera related dinoflagellate *Coolia monotis* on the chlorophyceae *Dunaliella salina* is described. Instead of mammalian cell lines which are commonly used in bioassays to detect celltoxicity, a green flagellate has been used. The morphological changes of *D. salina* as a reaction to the unknown toxin from *C. monotis* are quite similar to the effects which can be detected by mammalian cell lines as a reaction to the ciguatera toxin Maitotoxin (MTX). After addition of *C. monotis* cells to a culture of *D. salina*, the latter shows cell blebbing followed by lysis and cell rounding. The effect is Ca-dependent and can be inhibited by the Ca-channel blocker nifedipine. In contradiction to ciguatera toxins the effect is temperature and pH dependent. It terminates at temperatures above 40 C and has a pH optimum between pH 8-9.

CYANOBACTERIA BLOOM IN DAUGAVA RIVER DAMMED RESERVOIRS, LATVIA

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The first phytoplankton studies on Daugava River were undertaken in 1924, when the Riga sewage collection system was expanded. Dammed reservoirs were constructed in 1940 (Keguma HPP); 1962 (Plavinu HPP); 1975 (Riga HPP). In total 480 species of algae (65 blue-greens) were determined. First reports of cyanobacteria bloom in the cascade of HPP were stated in 1977. It is established that *Microcystis* settled to the bottom of reservoirs in October, after having dominated about three months. Enhanced temperature above 27^{o}C in July and August 1999 was the main reason for extensive cyanobacterial bloom by mixed composition dominated by *Microcystis* species (mainly *M. aeruginosa, M. wessenbergii, M.viridis*). Mean July ñ August temperature for Daugava reservoirs is 21-22.2^{o}C. The maximum of phytoplankton biomass more than 85 mg L^{-1} was recorded in August 1999 in the lower Reservoir of Cascade. Former observed summer maximum biomass was 24.7mg L^{-1} in July 1991. In September 1999 the *Microcystis* species were partly replaced by *Anabaena* spp. and *Oscillatoria* spp. Conspicuous large phytoplankton biomass constituted more than 92% of *M. aeruginosa* and dense surface scums of cyanobacteria have deteriorated water quality in Riga reservoir ñ one of the sources of the drinking water for Riga the Capital of Latvia, the Daugava River estuary and Riga Gulf. The results from September 1999 indicate that colonies of *Microcystis* associated with *Anabaena* spp., *Oscillatoria* spp., *Spirulina* sp. and unicellular diatoms are forming 1.5-3.0 cm thick green layer on top of the sediment. The data suggested that these high amounts of *Microcystis* in sediments would cause microbial activity ultimately leading to phosphorus leakage from sediments and potentially increasing hazard for Riga drinking water.

DETECTION OF CYANOBACTERIAL HAB SPECIES USING MOLECULAR APPROACHES: THE UTILITY OF NIFH AND 16S RRNA CHARACTERIZATION AND PROBING STUDIES

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Geographic expansion of toxic cyanobacteria is a serious threat to aquatic ecosystems. The detection and accurate identification of harmful cyanobacterial species is essential for monitoring the distribution and expansion of potential bloom formers. Molecular approaches are particularly useful due to the ability of these techniques to differentiate strains and detect HAB species at low densities. These approaches have been applied to two river systems with a history of cyanobacterial blooms. In the eutrophying, N-limited Neuse River Estuary, NC, USA, cyanobacterial species capable of N2 fixation may be periodically selected for bloom formation. The spatial and temporal distribution of the N2 fixing cyanobacterial population of the Neuse River Estuary was mapped by detecting the nifH gene, which encodes nitrogenase, through PCR. The nifH gene, and thus the genetic potential for these cyanobacterial bloom formers, was found to be present throughout the Neuse River Estuary during most of the year. Sequencing these PCR products revealed potentially toxic *Anabaena* sp. and *Anabaenopsis* sp. to be the most common nitrogen fixing cyanobacteria present. Deteriorating water quality due to harmful cyanobacterial proliferation also plagues the St. John's River System, FL, USA, which supplies drinking, irrigation and recreational waters for approximately 3 million people. The cyanobacterial community of the lakes in this river system was characterized using nifH and 16S rRNA. Molecular probes based on the DNA (nifH) sequences of toxic species can differentiate these from non-toxic strains. Identification of toxic cyanobacterial species at low densities is critical to permit management actions before these species reach bloom proportions.

OCCUPATIONAL EXPOSURE TO HARMFUL ALGAL BLOOMS: A PILOT STUDY

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The proliferation of harmful algal blooms (HABs) has intensified worldwide, causing deleterious effects to plants, animals and humans. In 1997, fish kills in North Carolina estuaries were attributed to a new organism, *Pfiesteria piscicida*. That year, the US Centers for Disease Control (CDC) and the affected States held a workshop to coordinate states affected by *Pfiesteria* and other *Pfiesteria*-like organisms (PLOs). Guidelines were developed to diagnose Estuarine Associated Syndrome (EAS) as a surveillance tool for persons with reported symptoms and exposure to estuarine waters.

Although *Pfiesteria* has not been found in Florida, a cryptoperidiniopsoid dinoflagellate (a PLO) has been associated with fish lesions in Florida. A cross-sectional study was performed to determine the association between occupational exposure to estuarine HABs and potential human health effects. Florida environmental workers, with extensive estuarine exposure, served as the study population creating 3 exposure groups: exposure to the cryptoperidiniopsoid dinoflagellate, exposure to only fish kills and/or fish lesions and a control group. After obtaining IRB approval, phone interviews were conducted using a revised EAS Questionnaire to inquire about reported exposure and symptoms.

The study population was a homogeneous group of 53 workers: 51 participants, 2 refusals; 41 (80.4%) ever exposed to fish events, 13 of whom were specifically exposed to the cryptoperidiniopsoid dinoflagellate. None of the participants met the CDC/States criteria for EAS. The subgroup (n=28) exposed only to fish kills/lesions did report more health effects than those exposed to the cryptoperidiniopsoid dinoflagellate or the control group. Although the trend was leaning towards significance, the comparison was not significant (p=0.08). These symptoms were associated with Florida Red Tide exposure. These environmentally exposed workers are an excellent population to follow for future investigations of occupational HAB exposure.

BIOMONITORING BREVETOXIN EXPOSURE IN MAMMALS USING BLOOD SPOT CARDS

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We have developed a method to monitor the exposure of mammals to brevetoxins. This sampling involves collecting whole blood, applying the blood to an inch diameter circle on a specially prepared blood collection card and allowing it to dry. This sample collection method has been widely employed for routine diagnostic and genetic testing of newborns. Collection instructions, cards and mailing envelopes are available without charge through our website at: http://www.chbr.noaa.gov/crpage.html. The blood spots are extracted in the laboratory and total brevetoxin activity quantified using high throughput receptor binding assay and specific brevetoxin congeners analyzed by liquid chromotragraphy-tandem mass spectrometry. Toxicokinetic characterization has been conduced with laboratory mice. Mice were treated with 180 μ g/kg brevetoxin-3. Whole blood was collected at time points between 0.5 and 24 hours of brevetoxin exposure and 0.1 ml was spotted on filter paper cards. Brevetoxin activity as determined by receptor assay increased between 0.5 and 4.0 hours and was decreased, yet detectable 24 hours after brevetoxin exposure. Tandem mass spectrometry was used to provide confirmation of brevetoxin-3. The mass spectrometry results paralleled those of receptor assay for time points between 0.5 and 4.0 hours exposure. However, brevetoxin-3 was not detected at 24 hours suggesting metabolism to another biologically active form of the toxin. We anticipate that this approach will provide a method to biomonitor for brevetoxins in living marine resources, protected species, and humans and are evaluating this biomonitoring method for other marine toxins as well.

DINOFLAGELLATES IN MANGROVE PONDS, PELICAN CAYS, BELIZE.

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Harmful and non-harmful dinoflagellates in the Pelican Cays coral reef ecosystem were examined, in an archipelago of mangrove islands situated on the top of an atoll-like coral reef, in the center of southern Belize barrier-reef lagoon. Mangrove fringed ponds in the Pelican Cays supported rich dinoflagellate populations. The six pond habitats selected were distinctive in its dinoflagellate species composition. Manatee Cay and Douglas Cay were the most species rich, with an unusual high number of oceanic species, whereas Elbow Cay exhibited benthic species. In Manatee Cay the taxa included 13 *Protoperidinium* spp., and 9 *Ceratium* spp.; in Douglas Cay 8 *Protoperidinium* spp. and 4 *Ceratium* spp.; and in Douglas Cay 7 *Gonyaulax* spp. and 3 *Prorocentrum* spp. The taxa of harmful species was the highest in the Manatee Cay: 5 *Prorocentrum* spp., 4 *Ostreopsis* spp., 3 *Dinophysis* spp., 3 *Gambierdiscus* spp. and 1 species each in the following genera: *Amphidinium, Cochlodinium*, and *Gonyaulax*, whereas the lowest number of species were in Lagoon Cay: *Gambierdiscus toxicus, Prorocentrum hoffmannianum* and *Pyrodinium bahamense* var. *compressum*, respectively. The Pelican Cays posses a diverse dinoflagellate assemblages of oceanic, planktonic, and benthic species including harmful species and provide important habitats for shallow-water microscopic flagellates.

DIFFICULTIES ASSOCIATED WITH PRODUCING AXENIC DINOFLAGELLATE CULTURES

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Several researchers have previously claimed to produce axenic *Alexandrium* cultures and used them in various studies, including investigations of bacterial effects on dinoflagellate toxicity. However, production of these cultures and ensuring their axenic status is a notoriously difficult task. This paper presents data detailing the effects of different antibiotic and chemical (EDTA, lysozyme and SDS) treatments on dinoflagellate microflora and highlights the pitfalls of these approaches. Particularly resistant isolates may be capable of assuming a cryptic form which can recolonise dinoflagellate cultures upon removal of antibiotics and chemicals. Results suggest that certain species within the bacterial consortia of both toxic and non-toxic dinoflagellates may constitute a crucial symbiosis. Therefore, the reduced bacterial community from treated dinoflagellate cultures has been characterised and compared with the original microflora in untreated cultures.

EUROPEAN APPROACHES TO MARINE TOXIN CONTROL

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A large number of EU coastal countries are affected by toxic events related to Harmful Algal Blooms. Toxic episodes due to Paralytic Shellfish Poisoning and Diarrhetic Shellfish Poisoning toxins have been registered from the 1950s and Amnesic Shellfish Poisoning toxins have been the cause of prolonged closures of shellfish production areas in the last few years. Recently, a new toxic syndrome caused by Azaspiracid and analogues has been identified in Irish mussels and might affect a number of EU countries. Monitoring programmes based on EU Directives have been implemented for marine toxin control in seafood. However, methods, performance criteria, and action levels have not been clearly established for toxin control, resulting in inconsistencies between EU countries, which are especially severe in the case of DSP toxin control. A network of European laboratories coordinated by a Community Reference Laboratory has been set up to create a forum of methodological and toxicological agreements and harmonize European seafood trade. Difficulties arise from the lack of pure toxins to perform toxicological studies and to develop and validate suitable analytical methods. European approaches to marine toxin control, agreements and activities on the field of harmonization of methods and limits are presented and discussed.

TOXINOLOGY AND TOXIN CONTENT OF *DINOPHYSIS ACUMINATA, D. ACUTA* AND *D.CAUDATA* FROM THE GALICIAN RIAS BAJAS.

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Dinophysis acuminata, D. acuta, and *D.caudata* co-occur during early autumn DSP outbreaks in the Galician Rías Bajas at the end of the upwelling season, and the contribution of these species to shellfish toxicity needed to be established. HPLC – FD analyzes of single cell isolates of these species during different events confirmed that *D. acuta* , with an OA:DTX-2 ratio of 3:2, is the source of DTX-2 toxin detected in Galician mussels, and that either *D. acuta* or *D. acuminata* can be the main contributor to the autumn toxic outbreaks. Preliminary results suggest that *D. caudata* has trace amounts of OA (<1 pg / cel). The present results confirm the large variability in toxin content in *D. acuta* and *D. acuminata* during different seasonal outbreaks, or even during the same outbreak in natural populations kept in the laboratory. The risk for human health of using a „*Dinophysis* index‰ as substitute information for the standard mouse bioassay in the early detection of diarrhetic shellfish toxicity is discussed.

LOCALISATION OF AZASPIRACID, A RECENTLY DISCOVERED SHELLFISH TOXIN, WITHIN THE BLUE MUSSEL MYTILUS EDULIS.

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Azaspiracid is a previously unknown and structurally novel phycotoxin found to be responsible for an outbreak of diarrhetic food poisoning associated with consumption of contaminated Irish shellfish in Europe in 1996. While azaspiracid was previously classified as a diarrhetic shellfish poison (DSP), it has recently been reclassified into a new poisoning category known as azaspiracid poisoning (AZP), because it has a number of unique properties that set it apart from the 'classic' DSPs, i.e. okadaic acid (OA), yessotoxin and the dinophysistoxins (DTXs). The response of mice to azaspiracid is characterised by hopping, scratching and progressive paralysis of the animal, leading to a quick death normally within 5-60 minutes, while mice injected with OA/DTX contaminated mussel extract die over a longer period of time following convulsions and prostration of the animal. This study describes the distribution of azaspiracid within the blue mussel. OA/DTX are known to be located exclusively within the hepatopancreas (HP) of the mussel, even to the extent that contaminated mussels with their HP removed are toxin-free and safe for consumption. Batches of mussels contaminated with azaspiracid, OA/DTX or both and greenshell mussels containing yessotoxin were dissected and their HP removed and pooled. The remaining flesh from each batch was also pooled and all fractions were extracted according to the Yasumoto protocol. The extracted solutions were then analysed via the mouse bioassay and by a novel cytotoxicity assay developed and optimised for the detection and discrimination of DSPs and azaspiracid. Only the HP fraction of the yessotoxin and OA/DTX contaminated mussels were toxic in the mouse bioassay, the flesh minus the HP had no adverse effect on the mice. A similar result was obtained with the cell bioassay. When azaspiracid positive samples were analysed a strongly positive result in both the mouse bioassay and the cytotoxicity assay was seen for both the HP and flesh minus HP fractions. When mussels containing OA/DTX and azaspiracid were dissected and analysed OA/DTX was only seen in the HP fraction while azaspiracid was seen in both dissected portions. This result indicates that the azaspiracid is distributed throughout the mussel and supports the observation that azaspiracid contaminated mussels take longer to depuriate than OA/DTX or yessotoxin contaminated mussels.

TOTAL LUMINESCENCE SPECTRA OF *PYRODINIUM BAHAMENSE* VAR *COMPRESSUM*

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Pyrodinium bahamense var *compressum* cultures and in situ samples collected from various parts of Manila Bay were analyzed by total luminescence spectroscopy in the ultraviolet and visible region. Synchronous fluorescence intensities from 200 to 800 nm were collected over various excitation wavelengths from 200 to 800 nm by using a Shimadzu RF-5301 double monochromator spectrofluorimeter. Data was plotted by using Surfer ú Surface Mapping System (Golden Software) on an IBM PC. Emission peaks were observed at 421-470 nm and at around 654 nm. Thus, a fingerprint of the organism was obtained from measured spectra. This data is to be the basis for development of a fluorescence LIDAR detection system for monitoring red tide blooms.

EFFECT OF UPWELLING PULSES ON EXCESS CARBOHYDRATE SYNTHESIS AS DEDUCED FROM NUTRIENT, CARBON DIOXIDE AND OXYGEN PROFILES

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The conservative chemical parameters "NO"; "CO" and "NCO" have been used to investigate the relationship between the upwelling intensity off the NW Iberian coast and the differential carbohydrate synthesis and utilisation in the water column. Differences between the observed vertical distribution of "NO" and "CO" and that expected from Redfield stoichiometry indicate that an excess of carbohydrate synthesis may occur in the surface layer when nutrients become depleted during upwelling relaxation periods. Excess carbohydrate synthesized in the upper layers is consumed in the subsurface levels. This spatial uncoupling between photosynthesis and nutrient uptake can be attributed to the presence of autotrophic migratory organisms like the photosynthetic ciliate *Mesodinium rubrum* and several dinoflagellates which produce carbohydrates in the upper, well illuminated layers, and take up nutrients in the sub-photic zone. Conversely, situations of intense upwelling are associated with production of organic matter following the Redfield ratio and with a decrease in carbohydrate synthesis. Deviations from Redfield stoichiometry found in other marine areas could be explained by processes comparable to those occurring in the NW Iberian upwelling, suggesting that vertical migration by photosynthetic organisms may play a greater role in the vertical transport of nitrogen and carbon than generally recognized.

SHORT-TERM AND LONG-TERM EFFECTS OF TOXIC DINOFLAGELLATES ON THE COPEPOD *ACARTIA CLAUSI.*

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In this study, several experiments were performed to determine the effects of cell toxin concentration, composition and toxicity of *Alexandrium minutum* on ingestion rate, egg production, hatching success and naupliar fitness of the copepod *Acartia clausi.* A combined of *A. minutum* and nontoxic algae (*Tetraselmis suecica, Isochrysis galbana* and *Prorocentrum micans*) was used as food. The strain of*A. minutum* used in this study (A1 1V) only contains gonyautoxins. Copepods ingested a higher amount of *A. minutum* cells as the concentration of toxic dinoflagellates increased, and in response to decreasing total food concentration available for the copepods. A positive relationship was obtained between *A. minutum* cells ingested by copepods and total toxin concentration per copepod. Only gonyautoxins were detected in the copepods. Egg production, hatching success and naupliar production were lower as copepods ingested a higher amount of toxic dinoflagellates. The negative effect of *A. minutum* on copepods was higher as *A. minutum* had a higher cell concentration of GTX1. Finally, the results obtained from nauplii incubated with *T. suecica* and *I. galbana* showed that, nauplii hatched from females fed nontoxic food (*T. suecica* and *I. galbana*) reached copepodite stage earlier, than those nauplii hatched from females fed with a combined of toxic (*A. minutum*) and nontoxic food (*T. suecica* and *I. galbana*).

PROTEIN SYNTHESIS INHIBITION ASSAY FOR CYLINDROSPERMOPSIN.

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The cyanobacterium *Cylindrospermopsis raciborskii* produces a toxin known as cylindrospermopsin. This toxin has been implicated as the causative agent of the Palm Island Mystery disease which led to the hospitalisation of 150 people in Queensland in 1979. More recently this cyanobacterium and other cylindrospermopsin-producing cyanobacteria have become increasingly of concern. They have been found in high numbers in drinking water reservoirs such as those supplying Brisbane. *C.raciborskii* has also been found in the more temperate waters of the Murray-Darling basin and in European freshwaters. It is known that cylindrospermopsin is a potent inhibitor of protein synthesis. We have utilised the rabbit reticulocyte lysate system to assay for protein synthesis inhibition. The response of cylindrospermopsin in this system has been compared to cycloheximide, a well known and widely used inhibitor of protein synthesis. Samples of *C.raciborskii* extract have been assayed for cylindrospermopsin content and the results correlate well with High Performance Liquid Chromatography (HPLC) data. We expect that the protein synthesis inhibition assay will be a useful tool for the rapid assessment of the bioactivity of C.raciborskii samples, as has been the case in our laboratory.

THE EFFECT OF DINOFLAGELLATE *ALEXANDRIUM TAMARENSE* ON EARLY DEVELOPMENT OF SCALLOPS

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The effect of a PSP producing dinoflagellate *Alexandrium tamarense* on early development of scallops (*Chlams farreri , Argopecten irradians*) has been studied. The results showed:1. *A. tamarense* could inhibit egg hatching, 36hEC50 was about 1,000cells/ml. The most sensitive stage of egg development to the toxic algae is before archenteron phase and the strongest impact was found when the toxic algae was at its early exponential stage. A conclusion was drawn from our experiments that the adverse effect to egg hatching was caused by some substances, non PSP origin, at the surface of cell membrane of *A. tamarense* after comparing the effect by other algae, *Phaeodactylum tricornutum* and *Chattonella marina*, as well as different fractions of *A. tamarense* cultures including cell contents, cell fragments, cell free medium and standard STX toxins. 2. Survival rate of scallop larva at early D-shape stage decreased significantly after exposed to *A. tamarense* for 6 days and its swimming ability could be inhibited in 2 hours, both at concentrations 5,000 cells/ml and above. 3. No obvious effect of *A. tamarense* was found on metamorphosis, mortality of eyespot larvae during 14-day experiment, but the growth of juvenile scallop was inhibited, with 14dEC50=5,000cells/ml. 4. Climbing ability of juveniles could be clearly reduces by *A. tamarense* in very short exposure, with 1hEC50=1,000cells/ml. The adverse effect of A. tamarense on early development of scallops especially on egg hatching indicated that *A. tamarense* bloom may cause decline of shellfish population and may have further impact on marine ecosystem.

DISTRIBUTION OF *PYRODINIUM BAHAMENSE* VAR. *COMPRESSUM* CYSTS IN THE SURFACE SEDIMENTS OF MANILA BAY, PHILIPPINES

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The mapping of cysts of toxic dinoflagellate in the sediment is a useful tool to determine the so called "seed bed" or "point source" where cysts are accumulated and can inoculate the water column to initiate blooms at any body of water. The first massive bloom of toxic dinoflagellate *Pyrodinium bahamense* var. *compressum* and its associated paralytic shellfish poisoning (PSP) was recorded in 1988 in Manila Bay. Progressively, it has become an annual recurrent event since 1990 in the Bay. Sediment samplings were conducted during two monsoon seasons (i.e., southwest monsoon on July-August 1993 and tradewinds on April 1996) to know the geographical distribution and concentration of *P. bahamense* cysts in the surface sediment of Manila Bay. Likewise, the grain size distribution of surface sediment is correlated with the accumulation and distribution of *P. bahamense* cysts in the Bay. Results showed that *P. bahamense* cysts are relatively low during the SW monsoon which ranged from 0 to 127 cysts/cm3 while during the tradewinds *P. bahamense* cysts are relatively high which ranged from 38 to 420 cysts/cm3. The geographical distribution of the cysts showed that the highest concentrations of 127 and 420 cysts/cm3 were constantly found in the western part of the Bay (in Limay, Bataan) during the SW monsoon and tradewinds, respectively. The eastern part of the Bay (off Manila South Harbor) also showed fairly high concentrations of the cysts that yielded 101 and 255 cysts/cm3 during the SW monsoon and tradewinds, respectively. These areas are also noted with relatively high mud silt contents of <22 μm size fine fraction. Most area of the Bay is characterized with high contents of <22 μm size fine fraction ranging from 45.5% to 90.5%, being relatively fine towards the interior and became coarser towards the mouth of the Bay. The abundance of cysts showed a clear pattern with the grain size distribution in the sediments particularly for fine fraction of <22 μm size that are accumulated in the western and eastern parts of the Bay. The western part of the Bay has been noted as the frequent origin of toxic red tide bloom in the Bay.

MICROCYSTIN DETERMINATION USING LC COUPLED WITH UV AND MASS SPECTROMETRIC DETECTION INCORPORATING SIMULTANEOUS CID-MS AND MS-MS

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Microcystins represent the most complex group of the known cyanobacterial toxins and they have been implicated in both animal and human intoxications throughout the world. The diversity of microcystins is apparent by the fact that over sixty toxins have so far been structurally elucidated. Microcystins are hepatotoxic and exert their activity by a potent inhibition of protein phosphatase 1 and 2A. A new liquid chromatography (LC) method has been developed which meets the requirement for both high specificity and sensitivity for microcystins by coupling diode array UV and mass spectrometric detectors. Six commercially available microcystins were separated by reversed-phase LC using an acetonitrile/water gradient. Microcystins have a unique b-amino acid moiety, Adda, and the a-cleavage of the methoxy group gives a fragment ion at m/z 135 which is characteristic of a microcystin and distinguishes these toxins from other classes of toxins. After UV detection, both CID and MS/MS experiments were carried out simultaneously using electrospray ion-trap instrumentation. The CID spectra for the microcystin standards, MC-YR, MC-LR, MC-LA, MC-LW, MC-LF, contained the molecular ion [M+H]+ at m/z 1045, m/z 995, m/z 910, m/z 1025 and m/z 986 respectively, MC-RR showed mainly the [M+2H]2+ ion at m/2z 520 and all gave the expected Adda fragment ion at m/z 135. MS/MS experiments revealed the [M+H-H2O]+ as a major fragment ion for each of the microcystins and an improved signal/noise was obtained compared with LC-MS. This method was applied to the analysis of lake water samples from Ireland that were positive by protein phosphatase assays. Both known and unidentified microcystins were confirmed in samples and the effect of clean-up steps on the integrity of microcystin analytes was also examined.

EVALUATION OF AN ELISA TECHNIQUE FOR THE DETECTION OF DOMOIC ACID IN THE SCOTTISH ASP MONITORING PROGRAMME.

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Monitoring of shellfish for amnesic shellfish toxins, principally domoic acid (DA), was implemented in Scotland in 1998 due to the inclusion of this toxin group in an amendment of the EU Directive on shellfish safety (EU 91/492). Routine testing involves evaluation of the DA content of shellfish using HPLC with either UV or DIODE array detection. Previously an ELISA technique, based on a DA antibody, was reported to be effective at detecting DA in shellfish. In this study its use in a monitoring programme to screen out negative samples, hence reducing requirements for more expensive analytical techniques, was assessed. The data generated will be presented and the feasibility of this approach discussed.

COMPARISONS OF THE LABORATORY AND KIT VERSIONS OF THE MOUSE NEUROBLASTOMA ASSAY, TO THE MOUSE BIOASSAY, FOR THE DETECTION OF PSP IN SHELLFISH.

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The current method for the detection of paralytic shellfish toxins (PSTs) in shellfish monitoring programmes is the mouse bioassay, however its use is becoming increasing unacceptable in several countries, including the UK, for ethical reason. This has led to the development of alternative methods, including a tissue culture assay, utilising mouse neuroblastoma cells expressing active sodium channels. This assay will detect sodium channel blocking toxins such as PSTs and can be conducted using published laboratory techniques or with commercial kits. Data from a trial comparing the laboratory and the kit versions of the assay to the mouse bioassay and a statistical interpretation of the data will be presented.

IMPORTANCE OF TEMPORARY CYST PRODUCTION IN THE POPULATION DYNAMICS OF *A. TAYLORI*

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Although temporary stages are common in dinoflagellates, their role remains unclear. Every year, A. taylori forms dense patches (10^6 cells/L) in La Fosca beach (Spain, NW Mediterranean), lasting for 2 months (July, August). One of the characteristics of the life history of *A. taylori* is the formation of temporary cysts. The hypothesis of the temporary cysts accumulated in the sediment play an important role in the sudden population increases observed once the adverse environmental conditioned have past is explored. Temporal changes in the abundances of temporary cysts in the sediment and, the in situ encystment and excystment dynamics of temporary cysts are presented. The daily in situ flux of the temporary cysts from water column to the sediment in the maintenance period of the bloom is of the order of 10^4 cysts/m2/day, whereas the flux of the daily excystment is 10^3 cysts/m2/day. Part of the temporary cysts in the sediment take more than one day to produce vegetative cells and remain viable in the sediment at least 4 days as a possible short-term population reserve. The data is analysed in view of the proposed hypothesis.

IMPLICATIONS OF ICHTHYOTOXINS IN THE POPULATION DYNAMICS OF *GYMNODINIUM MIKIMOTOI* :3D-MODELLING OF THE POPULATION CONFINEMENT IN THE PYCNOCLINE (BAY OF BISCAY – FRANCE)

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Blooms of *Gymnodinium mikimotoi* have deleterious effects on marine aquaculture stocks (fish and shellfish), on species recruitment (shellfish and probably fish) and possibly on marine flora and ecosystems. In stratified environments, populations are confined into very thin layers located in the pycnocline. The reasons for this confinement are yet unclear, the pycnocline being the only location either where this species can grow or where it can survive. We demonstrate here that pycnocline layer, when it exists, is the location where the maximum net growth rate is encountered, due to a lower mortality rate.Toxicity of *G. mikimotoi* is due to a labile exotoxin (20 min. half-lifetime). Synthesis of this exotoxin has allowed to determine the mechanism of action of this toxin: it inhibits in a non-specific way membranes ATPases. These enzymes are the energy source for ions exchanges at membranes. Biological targets are, therefore, incapacitated in their osmotic pressure regulation. The effect of these exotoxins have been studied in terms of stock losses, but never in terms of the effects on the ecology and the development of a bloom. The spatial scale of action in relation to degradation is of the order of few centimeters. Since individual cells have been observed to aggregate during the growth phase of the population, it is very likely that the population creates its own specific environment. Though less sensitive than their competitors, *G. mikimotoi* cells are sensitive to their own toxins. In still environments, like a culture, cells are able to avoid collision. However, in natural environments, collision rate of cells depend on the shear. A simple formulation of population growth implemented in the 3D-model of the Bay of Biscay has been used to simulate time-series in the Bay of Biscay (France) according to the following equation: with m: growth rate, hn: light intensity and g: shear. The zone of inoculation of the population was defined from different scenarios using analysis of trajectories. We present here the evolution of population growth along different transects, demonstrating the population confinement in the pycnocline.These results are discussed in the context of "species-of-interest" population dynamics approach.

AN OUTBREAK INVESTIGATION OF CIGUATERA FISH POISONING IN A CHICAGO RESTAURANT

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The index case contacted the Chicago Department of Public Health (CDPH) complaining of clinical symptoms associated with the consumption of amberjack served in a Chicago (Illinois) restaurant two weeks previously. CDPH initiated an outbreak investigation after 3 additional individuals, who complained to the restaurant, were identified as having similar clinical symptoms. The case definition consisted of acute onset of diarrhea or vomiting within 14 hours of eating amberjack at the restaurant, followed by the development of neurologic symptoms; associated cases were defined as sharing the amberjack with a case, followed by acute onset of diarrhea or vomiting within 14 hours in the absence of neurologic sequelae. The outbreak investigation follow up was conducted primarily using credit card receipts provided by the restaurant from persons who ordered amberjack from 7/27/99 until 8/3/99.

Of the 87 dining group tickets containing one or more orders of amberjack, 63 (72%) were paid by credit card and 24 (28%) by cash. All the credit card vendors were notified of the investigation. In addition, the local medical community was alerted through a CDPH newsletter concerning ciguatera. Of the 63 credit card payers, 44 (70%) contacted CDPH to be interviewed; 3 of 24 cash payers (11%) were also interviewed.

Of the 87 dining group ticket orders, 47 (54%) were interviewed, resulting in 19 cases and 2 associated cases of ciguatera. All the cases by definition continued to suffer from chronic neurologic symptoms. Of these 21 persons, 10 (48%) had sought medical care by the time of the interview, but only 2 (10%) had been diagnosed as ciguatera; 4 (19%) persons had sought specialist care with urology, infectious diseases, rheumatology, and odontology. Multiple diagnoses had been entertained including allergic reaction, multiple sclerosis, exacerbation of rheumatologic disease, and dental abnormality. Considerable monies had been spent on tests and treatments (including steroids and antibiotics).

In conclusion, credit card receipt tracking and communication with the medical community can be effective methods of outbreak investigation follow up for restaurant-associated foodborne illness. In addition, this outbreak illustrates the monetary and psychological burdens associated with misdiagnosis and delay in diagnosis of ciguatera in a non-endemic area.

BACTERIAL DIVERSITY IN TOXIC *ALEXANDRIUM TAMARENSE* BLOOMS OF THE ORKNEY ISLANDS AND THE FIRTH OF FORTH

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During toxic *Alexandrium tamarense* blooms in the area of the Orkney Islands and the Firth of Forth (Scotland) in 1998, two drifting experiments were performed and water samples were taken for molecular analysis of bacterial populations associated with the blooms. Genomic bacterial 16S-rDNA was amplified by PCR from three sample fractions (< 3 μm > 0,2 μm, < 10 μm > 3 μm < 100 μm > 10 μm). Bacterial community structure determined by DGGE (Denaturing Gradient Gel Electrophoresis) showed differences between the sample fractions but only slight variation during each drifting experiment. From two stations of one drifting experiment bacterial 16S-rDNA of the largest sample fraction (< 100 μm >10 μm, mostly dinoflagellate cells) was cloned and a number of 39 clones were sequenced. Additional, from the same samples bacteria were isolated on low nutrient media and the 16S-rDNA was also sequenced. >From 39 clones of the largest sample fraction, 25 clones were assigned to the α-subdivision of proteobacteria. Cultivation experiments on low nutrient media lead to the isolation of fast growing γ-subdivision proteobacteria. In contrast to other studies, the particulate sample fraction was dominated by α -subdivision proteobacteria. This sample fraction consisted of mostly viable dinoflagellate cells. Hence, our results indicate, that α -subdivison proteobacteria are closely associated to the blooms of toxic *Alexandrium tamarense*.

CARBON, NITROGEN AND PHOSPHORUS CONTENT OF SINGLE CELLS OF *DINOPHYSIS NORVEGICA* GROWING IN SITU, MEASURED USING A NUCLEAR MICROPROBE

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Elemental analysis (carbon, nitrogen and phosphorus) on 30 single cells and 6 thecae of *Dinophysis norvegica* collected from the Baltic Sea was performed using a Nuclear Microprobe (NMP). For each cell elemental maps were created and the cellular C, N and P content was obtained. Concentrates of *D. norvegica* cells (>95% purity) were simultaneously collected for toxin analyses and measurement of particulate C, N and P content by traditional methods for comparison. The traditional measurements of C, N and P yielded 2 times higher values per cell than the ones obtained by the NMP-method, nevertheless, the averages for the elemental ratios were nearly identical between the bulk measurements and NMP. Carbon per cell measured by NMP varied by a factor of 2.2 as could be expected in a growing population (267 – 583 pmol/cell). Nitrogen content varied from 6 to 38 and P from 0.6 to 2.1 pmol/cell respectively. The cells were divided into two groups: cells with N/P>16 (Redfield) (n=16) had a significantly higher average N content per cell than cells with N/P<16 (n=14). However, the two groups did not differ significantly in average P content per cell. Moreover, the average C/N ratio was significantly higher for the N/P<16 group than for the N/P>16 group, while the average C/P ratio did not differ significantly between the groups. Thus, in the studied population about half of the cells were nitrogen deficient to varying degree, while no cells could be characterized as P-deficient. The data shows that cellular nutrient status, especially cell quotas of nitrogen, can vary considerably within a population of one algal species growing under the same environmental conditions in situ.

HARMFUL ALGAL BLOOMS IN THE CHESAPEAKE BAY, USA: COMPARISON OF EVENTS

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Harmful algal blooms in the Chesapeake Bay and coastal bays of Maryland, USA, are not a new phenomenon, but they may be increasing in frequency, and in the types of bloom events. For example, during 1997, outbreaks of *Pfiesteria piscicida* were observed in several Chesapeake Bay tributaries, including the Pocomoke and the Chicamicomico River, while in 1998, *Pfiesteria* related events were not found, but massive blooms of *Prorocentrum minimum* occurred in the Pocomoke and other tributaries. In 1999, *Aureococcus anophagefferens* developed in the coastal bays in early summer in sufficient densities to cause a brown tide. Toxic *Pfiesteria* was responsible for fish kills at relatively low densities in the plankton population. *Prorocentrum minimum* and *A. anophagefferens*, on the other hand, were not toxic, but reached sufficiently high densities to displace much of the other phytoplankton and have ecological consequences. These 3 years differed in the amount and the timing of rainfall events, and in resulting nutrient loading from runoff from the largely agricultural land basin. Nutrient loading to the eastern tributaries of Chesapeake Bay have been increasing over the past decade. Much of this nutrient delivery is in organic form. The sites of the *Pfiesteria* outbreaks ranked among those with the highest organic loading of all sites monitored Bay-wide. The relative availability of the dissolved organic constituents DOC, DON, and DOP were also higher at sites experiencing *A. anophagefferens* blooms relative to those not. Dense blooms, like brown tides, pose other physiological challenges for the cells, and the ability to supplement photosynthesis with organic substrates may provide an advantage in maintaining blooms. For *P. minimum* and *A. anophagefferens*, urea is used preferentially over nitrate, and both increase their net growth rate in response to organic additions. *Pfiesteria* is a grazer, but has the ability to take up nutrients directly. Dissolved organic nutrients appear to play an important role in the development and/or maintenance of these HAB species.

A COMPARION OF HPLC WITH ELECTROCHEMICAL OXIDATION, HPLC WITH CHEMICAL OXIDATION, AND THE MOUSE BIOASSAY FOR THE ANALYSIS OF PSP TOXINS IN SHELLFISH.

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High performance liquid chromatography (HPLC) is a powerful tool for the analysis of PSP toxins found in shellfish. The PSP toxins are normally chemically oxidized to form a fluorescent derivative prior to detection. We have previously shown (Janiszewski and Boyer, Proceedings of the 5th ICHMA- Newport RI, 1993) that this derivative can also be formed electrochemically. Here we compare the results obtained from the HPLC using the traditional post column chemical oxidation (PCRS), HPLC with electrochemical oxidation (ECOS) and mouse bioassay using 40 toxic samples of giant scallop (*Placopectin magellanicus*) and 25 toxic samples of geoduck clam (*Panopea generosa*). Both shellfish types contained mostly gonyautoxins, with GTX-2,3 accounting for the bulk of the total toxicity. Distinct matrix effects were observed using the two shellfish types. Scallop samples presented a much dirtier matrix and required clean up on a solid phase extraction cartridge prior to analysis using the ECOS system. Both HPLC-PCRS (slope of the regression line = 1.01; R-squared = 0.94) and HPLC-ECOS (slope = 0.93; R-squared = 0.96) accurately predicted the mouse bioassay results using scallop samples. The geoduck samples were assayed without prior clean up on a solid phase extraction cartridge. For these samples, the PCRS accurately predicted the mouse bioassay results (slope = 0.92; R-squared = 0.98) whereas ECOS tended to underestimate the total toxicity as determined by the mouse bioassay (slope = 0.70; R-squared = 0.95). Most of the variation was contributed by samples whose total toxin content was less than 100 μg STX eq. per 100g FW. With proper sample preparation, both HPLC-PCRS and HPLC-ECOS provided a viable alternative to the mouse bioassay with the ECOS system simpler to purchase, maintain and operate. Detailed conditions for the set-up, use and operation of the HPLC-ECOS system with shellfish will be provided. Supported by funding from the New York Sea Grant Institute.

POLYMERASE CHAIN REACTION (PCR) BASED DETECTION *OF GYMNODINIUM MIKIMOTOI* AND *ALEXANDRIUM MINUTUM* IN FIELD SAMPLES FROM S.W. INDIA

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Species specific primers for PCR were constructed for two harmful species of dinoflagellates, *Gymnodinium mikimotoi* and *Alexandrium minutum*. The primers amplified a product of expected size from cultured cells of *G. mikimotoi* and *A. minutum* but did not yield any product with a wide range of other cultured algal cells, used as negative controls. The confirmation of PCR products was performed by restriction enzyme digestion of the products. The PCR method for detection of *G. mikimotoi* and *A. minutum* was applied on field samples. *G. mikimotoi* could be detected in 11 field samples by microscopy and all these field samples were positive by PCR. The cell counts of *G. mikimotoi* in the samples ranged from 306-2077 l^{-1}. *A. minutum* could be detected by microscopy in three different field samples which had cell counts ranging from 115 -1115 cells l^{-1}. *A. minutum* was detected by PCR from two of these field samples, but the sample which had a cell count of 115 l^{-1} *A. minutum* cells did not yield any product by PCR. Plankton samples that were negative by microscopy for any of the two species, were also negative by PCR. All the PCR products from field samples were confirmed by restriction enzyme digestion.

BIOCIDAL ACTIVITY OF HARMFUL ALGAE AND PEST CONTROL

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Cyanobacteria and microphytic algae (23 species), including toxic water bloom stimuli in Ukrainian waters and Black Sea coastal region were tested on some injurious organisms of different evolutionary level. As a result antibacterial, antihelminthic, deterrent and insecticidal characteristics of a number of cyanobacterial and microalgal (Dinophyta, Chlorophyta and Xanthophyta) species and strains as well as their natural associations can be ascertained. The most perspective variants of their application in medical and agricultural practice may be considered as selective and prophylactic means, and new biological preparations would be created on this base. Cyanobacteria and microalgae have displayed inhibitory action on vital functions (nutrition, growth, metamorphosis and reproduction) of some herbivorous insects ˆ Colorado potato beetle, fall webworm, lackey, gypsy, brown-tail and ermine moths. Their larval phases (especially junior instars) were the most susceptible to the action of algal metabolites, so the main part of insects (70.0-100.0%) was eliminated during these stages. The mortality of the following larval instars, pupae and imago was not so high, but metatoxic effect took place there. So a number of insects (for example, Colorado potato beetle) in natural communities was decreased because of a total mortality in all of phases (89.3-97.4%) and degradation of the second pest generation in 22.8-62.7% (in control variant without treatment this index increased in 717.3%). Applicable concentrations and doses of these new preparations did not bring any damage to the plants, entomophagans (coccinellids and carabids) and warm-blooded animals. Their biological activity and protective efficiency can be comparable with well-known microbial insecticides, and in some cases they are more preferable. In particular, it is very difficult for terrestrial organisms to be resistant to non-typical substances. There are several ways to obtain cyanobacterial and microalgal biomass for the practical employment. Some of them can use a natural material from harmful species with its subsequent treatment. Controlled methods of cultivation or sewage utilization in cattle-breeding complexes for nutrient substratum are acceptable for this purpose too.

QUANTITATIVE ANALYSIS OF REPRESENTATIVE TOXINS RELATED TO DIARRHETIC SHELLFISH POISONING BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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We optimized conditions for liquid chromatography-mass spectrometric analysis for the following ten representative toxins associated with diarrhetic shellfish poisoning: okadaic acid (OA), dinophysistoxin-1 (DTX1), 7-O-palmitoyl-OA, 7-O-palmitoyl-DTX1, pectenotoxin-1, -2, and -6, pectenotoxin-2 seco acid, yessotoxin (YTX), and 45-hydroxy-YTX. Emphasis was laid on securing good recoveries during clean-up steps. Toxins in the hepatopancreas of scallops (2g) were extracted with 90% methanol, transferred to chloroform by partition, and treated on a Sep-pak silica cartridge with acetone/methanol. Good recoveries were obtained except for 45-hydroxy-YTX, which was difficult to extract with chloroform by partition and thus was treated on a buffered Sep-pak C18 cartridge. [M-H]- ions were selected for monitoring carboxylic toxins, [M+Na]+ ions for PTX1 and PTX2, and [M-Na]- ions for YTXs. The method suites not only monitoring but also toxin accumulation and depuration studies.

NITROGEN OR PHOSPHORUS DEFICIENCY FAVOURS THE DOMINANCE AND TOXIN CONTENT OF HARMFUL SPECIES IN SUMMER BALTIC SEA PHYTOPLANKTON

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The aims were to investigate how the species succession and toxin production of a Baltic summer phytoplankton communities were affected by phosphorus, nitrogen and silicon deficiency versus sufficent conditions. A land-based enclosure experiment was performed in July 1998 with the Baltic Sea open waters. The phytoplankton communities were exposed to two levels of nitrogen, phosphorus and silicon deficiency and a non-deficient treatment. In deficient treatments, the daily added nutrients were added in order to give the same level of deficiency for either N or P concerning the Redfield ratio. For example the daily added N was 1.6 μM in the most N-deficient and P was 0.1 μM in the most P-deficient, i.e. N:P ratio of 16:1. The most N-deficient treatment was the one that forced the summer phytoplankton communities to the lowest levels of produced biomass. Although the daily added P was kept at the same N level of deficiency, the biomass in the P-deficient was higher than in the N-deficient treatments. Silicon limitation in the open waters of the Baltic Sea during the summer does not seem to exist, and this was proved by the initial concentrations of silicon (6.3 μM). Moreover, in the Si-deficient treatments the phytoplankton biomass were as high as in the N,P,Si sufficient treatment. In the most N-deficient treatment, the intracellular N-content was higher than the sum of all added nitrate suggesting that either nitrogen from organic source might have been used or N2-fixation was occurring. The N2-fixing toxic cyanobacteria, *Nodularia spumigena*, did grow well in all treatments, but as the other phytoplankton species, had the lowest biomass in the N-deficient treatments. The harmful dinoflagellate *Heterocapsa triquetra* wasin higher numbers in the N and Si deficient treatments showing the ability of this algae of mixotrophy. In all three deficient treatments, small flagellates increased in cell numbers. The diatom *Skeletonema costatum* was in very high numbers in all treatments where N was added in excess to the needs of the phytoplankton cells, including the most Si-deficient treatment Toxin (nodularin) content was higher in the N. spumigena cells which were grown in the most P-deficient conditions. Since phosphorus has a very high turnover rate in natural marine systems and our experiment shows that a shortage of nitrogen will produce less phytoplankton biomass, we hypothesize that it is unlikely that a reduction of P input to the Baltic will lead to lower biomass or toxicity/toxin production.

HUMAN HEALTH RISKS OF EXPOSURE TO *PFIESTERIA PISCICIDA*

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It is well established that harmful algal blooms are increasing around the world. As a result, there has been a been a concomitant increase in toxic algal species, algal toxins and potential risks to human health. Extant data suggests that the newly identified toxic dinoflagellate, *Pfiesteria piscicida,*or a morphologically related organism (MRO)) is neurotoxic in humans and laboratory animals. We studied 40 persons to date with presumed environmental exposure to these toxins with standard neuropsychological procedures. Select subgroups were followed over time and additionally studied with functional imaging (PET), structural cerebral imaging (MRI) and clinical neurologic procedures. Findings indicate that (1) acutely, exposed individuals have poorer performance on measures of divided attention and new learning than age, gender, education and occupationally matched controls, (2) the severity of cognitive alteration is proportionate to the intensity and duration of exposure, (3) the cognitive alterations appear to be reversible at three months post-exposure in 50% of the cases (4) one episode of intense exposure increases the probability of symptom recurrence with a repeated low level exposure and (5) functional alterations are identifiable on PET scan which persist at least 8 months post-exposure in highly exposed persons. We conclude that the neurotoxic effects of *Pfiesteria piscicida* (or MRO) are measurable and be persistent in some persons. Sponsored by funds from Maryland DHMH, Heinz Foundation and NIEHS-NIH.

BIO-OPTICAL CHARACTERIZATION OF THE DINOFLAGELLATE *GYMNODINIUM BREVE* AND THE DIATOM *THALASSIOSIRA WEISSFLOGII* IN OUTDOOR TANKS

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The bio-optical signatures of harmful algal blooms can be used to define ocean color satellite algorithms. We characterized the bio-optical properties of nutrient-replete cultures of the red tide dinoflagellate *Gymnodinium breve* and the diatom *Thalassiosira weissflogii*. We cultured large volumes (900L) of these organisms and performed measurements outdoors over a broad range of chlorophyll concentrations (0.1 to 30μg/L) characteristic of bloom progression. Measurements were made in unialgal tanks and in a mixed culture tank in which the proportion of the two species were varied but total chlorophyll remained constant at 15μg/L. The suite of measurements included HPLC photopigments, particle size distribution, in vivo spectral absorption and attenuation using WETLabs ac-9 and ac-100, filter pad/CDOM absorption, remote sensing reflectance, and backscattering. Preliminary results indicate that spectral absorption of the two species showed a high degree of similarity. Total scattering and backscattering spectra differed substantially between species in unialgal cultures and this difference was evident in the mixed culture tank. An experiment of this nature and scale has never before been successfully completed to our knowledge, and the results provide a valuable and unique data set with potential for ocean color applications.

RANGE OF *HETEROSIGMA AKASHIWO* (RAPHIDOPHYCEAE) EXPANDED TO INCLUDE CALIFORNIA

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Heterosigma akashiwo is a raphidophyte known to produce blooms which can result in massive fish mortality. During the spring of 1998 a red tide prevailed in San Pedro Bay, within the Los Angeles and Long Beach Harbors complex, centered at the mouth of the Los Angeles River. Based on light microscopy of living cells, the predominant organism was isolated and tentatively identified as being *H. akashiwo*. Previously this alga had never been documented in San Pedro Bay. To provide confirmation *that H. akashiwo* had become established in San Pedro Bay, the isolate identity was determined by sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region. Species determination was made by direct comparison to previously identified *H. akashiwo*. ITS sequence (isolate NIES 6). The result of this work was that there was complete sequence identity between the San Pedro Bay isolate and *H. akashiwo*. isolate NIES 6. This is the first confirmed documentation of *H. akashiwo* in Californian water, USA and an indication of continued expansion of *H. akashiwo*'s bloom range.

RRNA PROBES FOR IDENTIFICATION AND CHARACTERISATION OF MARINE PHYTOPLANKTON, WITH AN EMPHASIS ON TOXIC ALGAE

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A fast and reliable identification of nano- and picoplankton by light microscopy is often difficult because of the lack of usable morphological characteristics, whereas electron microscopy and biochemical methods, such as HPLC, are very time consuming. One possibility to solve this problem is to use taxon specific rRNA probes. For this purpose we searched an algal ribosomal rRNA sequence database for small unique regions to be used as probes to differentiate phytoplankton taxa. These probes were either labelled with Digoxigenin (DIG) and used in DNA dot blot experiments, or labelled with fluorochromes and used in whole-cell hybridisations with fluorescence microscopy or flow cytometric detection. Specific probes could be used over a broad taxonomic range from higher groups (i.e. dinoflagellates) to species level (i.e., *Prorocentrum micans*). These probes will be used in the EU MAST project AIMS for the development of an automated identification system for marine phytoplankton in combination with flow cytometry and artificial neural networks (ANN)and in the German national project (TEPS) for the development of an early warning system for harmful algal blooms. Illustrations are provided to highlight specific problems with dinoflagellate whole-cell hybridisations. This work was supported by the German BMBF TEPS 03F0161 and the EU AIMS MAS3-CT97-0080 projects.

PARALYTIC SHELLFISH TOXINS AND GLUTATHIONE S-TRANSFERASES IN ARTIFICIALLY INTOXICATED MARINE ORGANISMS.

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The metabolism of algal toxins by marine organisms is a field of emerging interest. In spite of numerous studies on the depuration of paralytic shellfish toxins (PSTs) from marine bivalves, little work has been conducted on the mechanisms of PST detoxification. Previous research indicates that exposure of salmon to saxitoxin by intra-peritoneal (ip) injection increased cytochrome P-4501A activity in the liver. Similar exposure experiments show PSTs to cause induction in the activity of the phase II detoxification enzyme, glutathione S-transferase (GST) in salmon (*Salmo salar*) livers and mussel (*Mytilus edulis*) digestive glands. Liver samples from salmon periodically injected ip over 21 days with saxitoxin and a toxic dinoflagellate extract showed elevated GST activity relative to controls. Immunodetection of GST isoenzymes by western and dot blotting with mammalian antibodies suggests that increased expression of GST protein may be the primary cause of the activity induction observed. Mussels were also exposed to doses of saxitoxin by intra-muscular injection. A small but significant elevation of GST activity was noted in the digestive glands of exposed groups relative to controls. This activity induction may be due to increased GST expression or enzyme activation. This work suggests that there may be a role for detoxification enzymes such as glutathione S-transferase in the detoxification and elimination of PSTs from these fish and shellfish models.

LONG-TERM AND SEASONAL DYNAMICS OF *DINOPHYSIS* SPECIES IN COASTAL AREAS AND THE OPEN BALTIC SEA.

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Long-term, high frequency studies of phytoplankton (0-20 m) in a coastal and open-sea area of the brackish northern Baltic Sea proper, were used to describe long-term and seasonal variability of *Dinophysis acuminata* and *D. norvegica*. In addition, we also studied their vertical distribution. The longest time series (1977-1998) are from two coastal stations, one exposed to the open Baltic Sea, the other from a large, less exposed bay (Himmerfjärden) enriched in nutrients. Since 1990 data are also available from an open sea station (The Landsort deep, 450 m the deepest part of the entire Baltic Sea).Our data show similar longˆterm behaviour of *D. norvegica* at the two coastal stations. This species was virtually absent in the seventies, increased during the eighties and was commonly present in high abundances during the early nineties. The open sea station behaved similar to the coastal station during the nineties. *D.acuminata* showed no clear long-term changes in abundances, but at the bay station, abundances were considerably higher compared to the other two stations. In contrast, abundances of *D. norvegica* decreased substantially from the open sea to Himmerfjärden Bay. We also found a clear seasonal as well as depth separation in the occurrence of *D. norvegica* and *D.acuminata*. Typically *D. norvegica* peaked later in summer than *D.acuminata* and mainly occurred close to the summer thermocline while *D.acuminata* occurred shallower, where light were more abundant.Despite large between year and station variability, these were not clearlyrelated to environmental conditions. However, the differences in distribution of *D. norvegica* and *D. acuminata* are likely explained by *D. acuminata* tolerating lower salinities.

ISOLATION OF NEW NEUROTOXIC SHELLFISH POISONS FROM THE NEW ZEALAND SHELLFISH, *AUSTROVENUS STUTCHBURYI*

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From December 1992 to March 1993 in New Zealand, it had been first noted that several cases of intoxication following ingestion of bivalves contaminated with toxins occurred. In the course of our study on this event, we had isolated a new brevetoxin derivative conjugated with taurine, named brevetoxin B1 together with Pbtx-3 from cockle, *Austrovenus stutchburyi,* and Pbtx-2 and 3 from oyster, *Crassostrea gigas*, collected at the off coast of north New Zealand. (1-3) From these results, it is clear that neurotoxic shellfish poisons (NSPs), brevetoxins were responsible for this food poisoning. New Zealand oyster accumulates brevetoxins produced by dinoflagellate, *Gymnodinium breve*, in the body. However, it is suggested that cockle metabolites brevetoxin B to brevetoxin B1 before storing in the body, whereas stores Pbtx-3 directly. As there are new NSPs, differing from known brevetoxins including brevetoxin B1, in cockle, we have been continuing an isolation study on these new toxins. We have isolated three new NSPs together with brevetoxin B1 and Pbtx-3 from 80% methanol extract of cockle, using chromatographies on columns of SiO2 , ODS and Sephadex LH-20, following by reverse-phase HPLC, successively. Their structural determinations are on going.

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ANALYTICAL DETECTION OF CIGUATOXINS-METHODOLOGY DEVELOPMENT

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Ciguatera poisoning., the result of ingestion of fish contaminated with one or more ciguatera effectors, is a relatively uncommon yet often debilitating form of ichthyosarcotoxism. For both philantropic and commercial reasons, it is of vital importance that fish carrying the transfused cyclic polyether ciguatoxin molecules be identified. Perhaps the penultimae goal associated with ciguatera detection research is the development of a method, which by virtue of its sensitivity and selectivity can be considered a benchmark method. Clearly, rapid throughput tests are dependant upon such a method for their realisation and validation. Gradient reverse phase HPLC-tandem mass spectroscopy (HPLC/MS/MS) has been shown capable of the desired sensitivity and selectivity for pure Pacific ciguatoxin-1 (P-CTX-1) and P-CTX-1 spiked into fish flesh extract; however, at this stage, sample preparation is slow and cumbersome. Results indicate that the employed HPLC/MS/MS method requires further optimisation to achieve increased sensitivity and selectivity for P-CTX-1. Utilisation of 1 μM ammonium acetate buffer in the mobile phase yields some increase in selectivity for P-CTX-1 as [P-CTX-1 + NH4]+. HPLC/MS/MS sensitivity has been improved by adjustment of the declustering potential decreasing the formation of [P-CTX-1 + NA]+., and by increasing the energy of the selected ions as they reach the collision gas chamber, allowing maximum fragmentation of the analyte. Extraction methodology has been concurrently evolving: a triphasic extraction asystem, comprising aqueous 2.5 M sodium chloride/acetonitrile/hexane, can quickly extract 10-12 mg crude lipid material from 5 g fish, a 500 fold preconcentration. Solid phase extraction (SPE) using Florisil allowing 82% recovery pure P-CTX-1, routinely causes a 10-fold reduction of lipid extract reducing matrix which interferes in the HPLC/MS/MS analysis. Lipid extracts at the levels reported cause matrix suppression of P-CTX-1 signal when analyses using HPLC/MS/MS. A radio-labelled ligand binding assay, involving displacement of tritium labelled brevetoxinby ciguatoxin from their shared binding site, is allowing assessment of P-CTX-1 partitioning within the current extraction procedure.

LESSONS OF ALGAL POPULATION DYNAMICS IN HABS FROM SIMULATION ANALYSIS

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Expression of algal population dynamics is at the base of Models of HABs. Increasing evidences from experiments suggest specific physiology of causative species is necessary for exceptional events of HABs, to incorporate parameters of physiology and life-history in HAB models draws an extensive attention in modelists. This contribution aims to detect determination of these parameters by modeling. Nutrient uptake is expressed in Droop's equation, simulations indicate that carrying capacity for algal cell number is determined by minimal cell quota for cell division and concentration of limiting nutrients. Nitrate concentration in sea water recorded in literature all are sufficient to support a cell number over HAB criteria. Therefore, the key question confronting from an world-widely increasing HABs is not what triggers HABs but what changes classical diatom blooms into Dinoflagellate Blooms. As exceptional events, HABs usually last a relatively short period, one or two weeks. Only a high cell division rate is insufficient for the short period blooms, simulation results that are sensitive to temperature dependence of algal growth rate suggest that causative species of HABs should have an unique temperature dependence in physiology. At the end of HABs, nutrient concentration is not exhausted, why algae stop growing seems to be the most curious question related to disappearance of HAB. Simulation results do not support grazing pressure assumption, but nonlinear death rate of density dependence as a cause. Virus or bacteria seem to be a possible access. In a mono-specific bloom, annual pattern of population size is usually characterized by a single peak. When cyst formation and germination are considered, simulations show population dynamics are characterized by two peaks at least, one occurs in spring and another in autumn. Two cyst forms: quiescent and dormant lead to different annual cycles of population dynamics.

NUTRIENT STOICIOMETRY OF A *GYMNODINIUM BREVE* BLOOM: WHAT LIMITS BLOOMS IN NUTRIENT STOICIOMETRY OF A *GYMNODINIUM BREVE BLOOM*: WHAT LIMITS BLOOMS IN OLIGOTROPHIC ENVIRONMENTS?

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Blooms of the Florida red tide dinoflagellate *Gymnodinium breve* occur in oligotrophic west Florida shelf waters which are depauperate of both dissolved inorganic nitrogen (N) and phosphorus (P). The nutrient stoichiometry of a small (maximum *G. breve* concentration 1.1 x 106 cells l^{-1}), nearshore bloom which persisted off Sanibel Florida from November 1998 to February 1999 was examined using particulate nutrient measurements from both the Florida shelf area and the bloom itself. The bloom was characterized by unique C/Chl, C/N and N/P ratios which differed from surrounding (ie. nearshore without *G. breve* present, offshore (~100 km) surface waters or the deep chlorophyll maximum (DCM)) areas. Chlorophyll a was only slightly elevated in the bloom (3.6 μg l-1) compared with both offshore and nearshore waters (0.16 μg l-1 to 1.87 mg l-1), however C/Chl ratios of the bloom ranged from 84.6 to 296.1 while those of nearshore, offshore and DCM areas ranged from 104.5-692.0, 355.9-2529.5 and 84.6-421.6 respectively. The variability and large range of C/Chl values may be due to variability in detrital contribution to carbon or extremely low chlorophyll concentrations. Both low inorganic N and P concentrations and stoichiometric calculations of N and P requirements based on observed bloom biomass suggest that sufficient P and insufficient N was present to support the bloom. However, N/P ratios for both bloom (15.9-46.6) and non-bloom (21.9-57.36) areas were consistently greater than Redfield thoughout this period, suggesting an unidentified N source was available to both bloom and shelf phytoplankton populations on the west Florida shelf.

PARTIAL BIOCHEMICAL CHARACTERIZATION AND TOXICOLOGICAL EVALUATION OF THE DINOFLAGELLATE *EXUVIAELLA LIMA* ISOLATED FROM EL PARDITO ISLAND IN BAJA CALIFORNIA SUR, MEXICO.

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In August 1997, the marine dinoflagellate identified as *Exuviaella lima* (or *Prorocentrum lima*) was collected from El Pardito Island in Baja California Sur, Mexico, after the report in that zone of ciguateric-type human intoxication. *E. lima* was isolated in the laboratory with the methods of micropipete, serial dilutions and agar petri dishes. *E. lima* has been reported to produce the diarrheic toxins: okadaic acid (OA), and dinophysistoxins (DTX-1, DTX-2, DTX4, DTX-5) that are associated with the ciguatera complex. *E. lima* was cultured in ES, K and f/2-Si medium, 12:12 light/dark cycle, and 22 C with constant stirring. The biomass was collected at the beginning of its stationary growth phase. Extraction and quantification of fatty acids (GCMS) and pigments (HPLC) was done. The major fatty acids (in order of decreasing abundance) were: 16:0 and 14:0 in the three culture medium; 18:4 was detected in f/2 and K, and 18:00 in ES; 20:5(n-3) and 22:6(n-6) in f/2, ES and K; 20:4 was detected only in f/2. The pigments profile consisted of chorophyll a, perydin, chlorophyll c1.c2, alloxanthin, zeaxanthin, b-carotene and fucoxanthin. Mouse bioassay, *Artemia* assay were done in order to evaluate the toxicity of a crude extract, resulting positive in both cases. Antimicrobian test was done in yeast showing a growth inhibition in the strain tested. TLC was used to identify the presence of toxins: OA and DTX-1. This study constitute the first isolation, culture, biochemical characterization and evaluation of the toxic dinoflagellate in Mexico.

THE USE OF NON-ANIMAL ASSAYS WITHIN A BIOTOXIN MONITORING PROGRAMME

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In England and Wales an algal biotoxin monitoring programme is undertaken which involves the monitoring of water samples for potentially toxic algae and shellfish flesh for Paralytic, Diarrhoeic and Amnesic shellfish poisons (PSP/DSP/ASP). The detection of PSP and DSP in shellfish is by mouse bioassay. To help reduce the number of animal assays performed work was undertaken to establish non-animal assays for PSP/DSP detection. Both cytotoxicity and commercial ELISA assays were tested and incorporated into a shadow biotoxin monitoring programme to test the reliability and feasibility of using these tests during an active monitoring programme. Potential application of these techniques within the programme are discussed.

SEQUENCE COMPARISONS OF TOXIC AND NON-TOXIC *ALEXANDRIUM TAMARENSE* ISOLATES FROM UK WATERS

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Paralytic Shellfish Poisoning (PSP) is a problem worldwide and in many cases is linked to the occurrence of toxic algal cells of the genus *Alexandrium* (Dinophyceae) in the marine environment. In the UK the species which has been linked with PSP is *A. tamarense.* Both toxic and non-toxic strains of this species have been found in the UK but these strains cannot be differentiated morphologically. The ability of certain species of *Alexandrium* (including *A. tamarense*) to produce toxins appears to correlate with phylogenetic lineage. North American and temperate Asian lineages consist exclusively of toxic strains and the western European line consists solely of non-toxic strains. To date, all toxic strains of *Alexandrium* found in UK waters can be assigned to the North American lineage based on their rRNA gene sequences, and it has been suggested that these strains are introductions, whilst the non-toxic strains are native to UK waters. We have conducted studies to determine if the correlation between toxicity and genetic lineage holds true for *Alexandrium* strains isolated in UK waters. Sequence and toxicity data for *A. tamarense* isolates will be presented.

TOXICITY OF *CHATTONELLA OVATA*, A NOVEL SPECIES OF *CHATTONELLA,* TO RED SEA BREAM *PAGRUS MAJOR*

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Recently an axenic cell line of a novel species *Chattonella ovata*, morphologically similar to harmful *C. antiqua*, was established. It could grow to the cell density of 5 x 105 cells/ml in modified SWM-3 medium at 20?C?(under an illumination of 45μE m^{-2} s^{-1} with a 12h light: 12h dark photocycle) This finding suggests that this organism has potentiality to form red tides in natural seawater. In the toxicological study performed at 20 C, all the red sea breams *Pagrus major* of 5 fish tested were killed with 5.1 and 6.8 x 10^3 cells/ml of this organism within 4 hours, although they were alive with 2.8 x 10^3 cells/ml. DO values of the seawater in the experimental groups did not decrease throughout the experiment. In the histological analysis, high frequencies of edema and hyperplasia were observed in the second gill lamellae of the fish exposed to the experimental red tides of *C. ovata*, and hyperplasia was observed in the lamellae of the fish killed by this organism. But, no extra-ordinal production of mucus was found in the tissue. The alteration of the gill tissue may be the cause of the fish death by *C. ovata.* These findings revealed that this organism has high toxicity to the fish. Therefore, we must consider the possibility of this organism to become one of the harmful algae in future.

TOXINS IN CYANOBACTERIAL MATS FROM MELTWATER PONDS ON THE MCMURDO ICE SHELF, ANTARCTICA

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Cyanobacteria are known to produce hepatotoxins, the functional and ecological role of these toxins, however, remains largely unclear. The toxic properties of cyanobacteria collected in Antarctica were investigated to determine whether toxin-producing species can also be found under these environmental conditions. Samples were collected from ponds near Bratina Island on the McMurdo Ice Shelf, Antarctica in the summers of 1997 through 1999. These ponds form from melt-water in the Antarctic summer and are colonized by benthic cyanobacterial mats. Oscillatoriales, *Nodularia* sp., and *Nostoc* sp. constituted the major taxa in freshwater ponds, while Nostoc sp. was missing from brackish and saline ponds, as determined by light microscopy. Samples were taken from either floating, submerged or benthic mats, subsequently frozen, freeze-dried, and extracted for in vitro toxicity testing. The phosphatase-inhibiting activity of the extracts was tested using rape seed phosphatase and 32P-phosphorylase a as substrate with microcystin-LR as the standard. The cytotoxic properties of the extracts were investigated using rainbow trout hepatocytes determining MTT metabolism and trypan blue dye exclusion. The results show that all cyanobacterial extracts have some phosphatase-inhibiting activity, of which approximately half had significantly greater than 50% inhibiting activity. Cytotoxic properties, apparently independent of the phosphatase inhibiting activity, were also detected. Toxic strains of cyanobacteria can therefore also be found in Antarctica and this finding may lead to further insight into potential ecological roles of cyanobacterial phosphatase inhibiting toxins.

THE POTENTIAL OF HARMFUL ALGAL BLOOM IN THE ARCTIC OCEAN AND OTHER HIGHER ARCTIC WATERS

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A recent survey on the phytoplankton composition in the Arctic Ocean (70-80^{o}N, 160-180^{o}W) revealed that dinoflagellate comprises the highest percentage of phytoplankton in surface water. The situation is different from that in Bering Sea and Baffin Sea where diatoms and golden-brown algae are usually the most dominant species during summer time. Taxonomic analysis confirmed that *Alexandrium catenella* and *A. tamarense* appeared in very high concentration at 5 M to 15 M below the sea surface. Their concentrations might be as high as 1650 cell/ml-1 at certain sampling stations. The results coincided with events of PSP in Alaska in recent years and the presence of low level PSP toxins in shellfish samples collected from the northern Greenland. Instead of settling at the bottom sediments, cysts of *Alexandrium* Spp. often appeared in the water at 15-20 M below the sea surface of Arctic Ocean. Some of the cysts are found trapped beneath floating ices. The phenomenon is closely related to the special climatic characteristic of the Arctic Ocean where ices are formed and deformed in a relatively rapid rate. The nutrient levels in surface water are analysed, with discussions over the potential and implications of harmful algal bloom in the arctic waters near Alaska and the NW Territories of Canada.

THE HARMFUL ALGAL BLOOMS IN HONG KONG WATERS – WHERE ARE THEY FROM?

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Hong Kong is one of the most frequent sites of Harmful Algal Blooms in the world. There have been over 500 algal bloom incidents recorded in Hong Kong Waters during the past 25 years, with 62 causative species responsible for these events. Why are there so many HABs in Hong Kong Waters, and where are they from? Guangdong and Hong Kong share the same coastal waters in Southern China, and each one's HABs affect the other as shown by the example of the historical record of the 1998 bloom. Based on the available data, literature and our own studies, we compared the HAB events and causative species recorded in both Hong Kong and Guangdong waters. The results reveal that of the 67 records for HABs from January 1980 to May 1998 in southern Chinese waters, 24 blooms occurred in the same time slot in both Guangdong and Hong Kong waters. Of these, 16 included Noctiluca scintillans, which is the most frequently recorded HAB species in both waters. Of the remainder, six were *Gymnodinium/Gyrodinium* species, and the other two were *Prorocentrum sigmoides* and *Chattonella marina*. This overlap of bloom occurrences underlines the need to consider HABs in Hong Kong in the context of a contiguous water mass, with flow moving in both directions between Hong Kong and Guangdong waters. Dapeng Bay (called Mirs Bay in Hong Kong) is where the most frequent HABs have taken place

DOMINANT SPECIES FROM SAM XING WAN, SAI KUNG DURING THE 1998 MASSIVE FISH KILLING RED TIDE IN HONG KONG

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A massive fish killing red tide occurred in Hong Kong from mid-March through mid-April, 1998. Weekly samples collected from a permanent station in Sam Xing Wan, Sai Kung were studied by light and scanning electron microscopy. *Gyrodinium digitatum*, which is believed to have caused the fish kill, is a new species. It is morphologically closely related to *Gymnodinium mikimotoi*, a fish killer firstly described from Japanese waters. Its highest cell density recorded from this station reached $2x10^5$ cell/L of water. *Alexandrium* spp. ($2.8x10^4$ cell/L), *Gymnodinium catenatum* ($6x10^3$ cell/L), *Gymnodinium sanguineum* ($3X10^5$ cell/L), *Prorocentrum triestinum* ($6x10^5$ cell/L), *Chaetoceros* spp. ($2x10^5$ cell/L) , *Pseudo-nitzschiapseudodelicatissima* ($6x10^5$ cell/L), *Skeletonema costatum* ($1.1x10^6$ cell/L), *Thalassiosira* spp. ($1.7x10^5$ cell/L) and *Scrippsiella trochoidea* ($8x10^4$ cell/L) were all dominant or relatively dominant species during this period. One other species, which was revealed by scanning electron microscopy, is probably an unidentified inoflagellate species. Morphological features will be presented, especially on the new ones.

TOXIC CYANOBACTERIAL BLOOMS IN THE LAKE OF BOURGET (FRANCE)

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Since two years, the lake of Bourget (France) which is used for the water supply to several agglomerations, has known problems with cyanobacterial blooms. A first bloom implying *Planktothrix rubescens*, was observed from November 1998 to March 1999. In less than two weeks, the cell number increased to 20.000 cells ml-1 and the filament dry mass to more than 1.4 mg l-1. The vertical distribution of the filaments was homogeneous above the thermocline, through the depths of 0-45 m, and the horizontal distribution was also homogeneous on the lake. At this time, more than 80 % of the biomass was constituted by *P. rubescens*. A first decrease of the cell number per milliliter was observed at the end of December. This decrease fits to a drop of the thermocline. In January, the temperature of the water mass of the lake was homogeneous (from 0 to 140 m) and the density of cyanobacterial was also homogeneous from the bottom to the surface (10.000 to 15.000 cells ml-1). At the middle of February, a second decrease was observed until March, at the end of the bloom. In July 1999, a strong increase of the cyanobacterial biomass was observed with 10 meters of depth. Two species were present at the beginning of the bloom: *P. rubescens* and *Aphanizomenon flos-aquae*. From July to the middle of September, the cyanobacteria were principally located in the metalimnion, between 10 to 15 meters from depth. After that, the vertical distribution of cyanobacteria became more homogeneous above the thermocline and *Pseudanabaena* sp. and *Oscillatoria limnetica* substituted *A. flos-aquae*. Toxicity of these blooms was analyzed by HPLC. Two forms of the microcystin-RR were mainly observed. The amount of microcystin per milliliter was correlated to the number of cells per milliliter and more than 4 μg/l of microcystin was observed after cell extraction, in water at different times during the blooms. The role on the dynamic of the blooms, of several factors like the interspecific competitions and the potential incidence of these blooms on the human health would be discussed.

ENHANCEMENT OF THE GROWTH OF MURINE COLON CANCER PRECURSORS BY MICROCYSTIN-CONTAMINATED DRINKING WATER

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Microcystis aeruginosa is a common cyanobacterium which produces toxic cyclic peptides called microcystins, potent hepatotoxins that have been implicated in tumour promotion in skin and liver. Cyanobacteria grow in many drinking water sources, so there is the potential for exposure of human water consumers. We have performed a series of studies to assess the potential for tumour promotion and carcinogenesis in mankind by cyanobacterial toxins such as the microcystins. In the present investigation, the model used was the azoxymethane (AOM)-induced aberrant crypt foci in the male C57BL/6J mouse colon. Tumours were initiated in the colons of mice by repeated AOM treatment and then drinking water containing *Microcystis* extract was supplied and continued for 212 days. The content of microcystins in the drinking water was determined by mouse bioassay, High Performance Liquid Chromatography (HPLC), capillary electrophoresis and protein phosphatase inhibition. The doses employed were approximately 0, 400 and 700 μg microcystin-LR equivalent/kg bodyweight/day. Although growth was initially reduced in the AOM dosed mice, no significant long-term effect of microcystin on growth rate was seen. At the end of the exposure period, a detailed post-mortem examination was performed followed by investigation of effects on haematology, serum chemistry and organ histopathology. A microcystin dose-dependant decrease in albumin concentration and increase in alkaline phosphatase activity in sera indicated sub-lethal toxicity. Measurement of aberrant crypt foci showed a significant (P less than 0.005) microcystin dose-dependant increase in both median and mean focus area. This investigation has provided the first evidence of stimulation of preneoplastic colon tumours by microcystin.

BLOOMS OF DINOFLAGELLATE *GYMNODINIUM C.F. MIKIMOTOI* IN KUWAITI WATERS

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The marine environment of the state of Kuwait has observed a massive fish kill mainly in mullets (*Liza macrolepis*) from 17 September 1999.Five days latter, water coloration was observed in Al-Beda,a coast south of Kuwait Bay. The bloom was due to the dinoflagellate *Gymnodinium* spp; other species detected in the bloom including *Prorocentrum micans, Dinophysis caudata* and *Gyrodinium spirel*. In the4th October the bloom was spread in Kuwait Bay. This time was associated with another fish kill, particularly in the cages of aquaculture sea bream (*Sparidentex hasta*), it was estimated that 100 tons of fish were killed .The bloom species was *Gymnodinium c.f. mikimotoi*. Four stations were established at various patches, they were samples for plankton analysis, water quality and chemical analysis. The bloom was mainly attributed to *Gymnodinium c.f. mikimotoi*, with cell counts ranged from 2678000 to 7627000 cells/l. The *G. mikimotoi* bloom was succeeding by the bloom of photosynthetic ciliate *Mesodinium rubrum*. The main objective of this report is to share Kuwait experience of dealing with the first harmful algal blooms in its territorial water and finding the environmental conditions .In addition to assistant and support Environment Public Authority (EPA) received from the national, regional and international communities.

TOXIN PROFILE OF WILD AND CULTURED CELLS *ALEXANDRIUM MINUTUM* IN TAIWAN

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The wild dinoflagellate *Alxandrium minutum* was collected from the coastal area and aquaculture pond in Yapu Aqueduct, southern Taiwan from February 1996 to April 1997. It was found the toxic dinoflagellate *A. minutum* appeared from December to March. HPLC analyses showed that the toxins from the coastal area were GTX 3 (96.8%), GTX 2 (3.2%), GTX4 (0.2%), STX (0.1%), and neoSTX (0.1%), while toxins from the aquaculture pond were GTX 3 (96.3%), GTX 2 (3.4%), GTX4 (0.3%), and GTX 1(0.1%). On the other hand, *A. minutum* T1 was isolated form an aquaculture pond in February 1996, and grown under various environmental and nutritional conditions. The optimal environmental condition for cell growth and toxin production of *A. minutum* T1 was as follows: temperature 25C, pH 7.5, light strength 6 x 10^3 lux, and salinity 15 ppt. The optimal level of nutrients supplemented in the 50% natural seawater medium for those of *A. minutum* T1 was as follws: phosphate 0.002%, nitrate 0.01%, cupric ion 5.0 ppb, ferric ion 270 ppb, and humic acid free. HPLC showed *A. minutum* T1 contained GTX 1-4 only. Among these components, toxins 1,4 were the predominant compounds throughout the growth circle when the cells were grown in the optimal environmental and nutritional conditions. But toxins GTX 2,3 increased when the cells were cultured at high salinity medium.

THE AMOUNT OF *HETEROCAPSA CIRCULARISQUAMA* CELLS TRANSFERRED WITH SHELLFISH CONSIGNMENT AND THE POSSIBILITY THEY WILL BEGIN TO RESIDE IN NEW CULTURE AREAS

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The harmful dinoflagellate, *Heterocapsa circularisquama*, has been expanding rapidly in the coastal areas of western Japan since 1988, causing mass mortality of shellfish. Since a large amount of pearl oysters and oysters are frequently transported to the many culture grounds within Japan, we examined the amount of cells transferred together with the shellfish consignments and the possibility that they will begin to reside in new areas. Pearl oysters (one year old and two years old) and oysters (spats and two years old) were exposed to *H. circularisquama* (16 – 10,000 cells/ml) for 10 minutes. Thereafter, these samples were permitted to stand in the laboratory for 4 hours. Each sample was immersed individually in fresh culture medium and the *H. circularisquama* cells which were vomited from the shellfish were counted. Furthermore, pearl oysters or oysters sent from embayments such as Ago Bay and Hiroshima Bay to Kyushu University within 24 hours were immersed in fresh medium. Those immotile cells which were vomited were inoculated in intact seawater medium containing phytoplankters to confirm the possibility that they will begin to reside in new areas. The results demonstrated that 1) the number of *H. circularisquama* cells which existed in the shellfish increased in logarithmical proportion to the cell density to which the shellfish were exposed, 2) immotile cells of *H. circularisquama* were found in shellfish collected from the sea areas where motile cells had been obsrved, and 3) immotile cells reverted to motile cells when diatoms did not grow in the intact seawater medium, although they remained immotile when diatoms were growing in the medium.

SMALL INTESTINAL INJURIES IN MICE CAUSED BY A NEW TOXIN, AZASPIRACID, ISOLATED FROM IRISH MUSSELS

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A new type of food poisoning resulting from ingestion of mussels from Ireland occurred in the Netherlands in 1995 and then reocurred in Ireland in 1997. This type of poisoning have been reported in Sweden, Scotland, Finland and France, also. As the causative agent, azaspiracid, was isolated in pure form and revealed to have an entirely new structure, in vivo studies with mice were carried out to elucidate the pathological injuries caused by the toxin. By peros administration, other than pathological changes in lymphoid issues and fatty changes in the liver, the small intestinal villi were the target organ. This report focused on the changes in the small intestine. First, the toxin caused necrosis in the laminapropria then atrophy of the laminapropria, and finally erosion of villi.

ION-EXCHANGE SEPARATION OF PSP TOXINS FOR HPLC DETERMINATION WITH DIFFERENT DETECTION

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One of the most important and dangerous intoxication from seafood with algal toxins is paralytic shellfish poisoning (PSP) caused by a group of 18 neurotoxins. The closely related charged molecules act as potent sodium-channel blocker and cause paralytic symptoms such as respiratory insufficiency, in serious cases with fatal results. The broad toxicity range of different PSP toxins in a line with diverse PSP toxin profiles in dinoflagellates and the possibility of biotransformation of PSP toxins in marine organism has challenged analytical chemists to development accurate and reliable analytical methods. The separation of charged and highly water soluble organic compounds requires HPLC methods based on phosphate buffered ion-pair chromatography on reversed phases. Additionally, PSP toxins do not show any fluorescence or native UV activity and they are normally detected after chemical oxidation and conversion into fluorescent derivatives. The combination of ion-pair reagents and oxidation chemicals results in many disadvantages. Ion-pair former, phosphate buffer and derivatization chemicals prevent the on-line coupling of the separation technique to an MS spectrometer. The presented method is based on ion-exchange separation of PSP toxins using a cation and an anion exchange column. The separation of important toxin pairs (e.g. STX/NEO, GTX2/GTX3, and C1/C2) is easily achieved. Some advantages are evident. The lower concentration of buffers and missing ion-pair reagents allows the replacement of the complicated post-column oxidation unit by a simple electrochemical cell. The ion-exchange separation in combination with electro-chemical oxidation offers a cheap way for standard production, as the up-scaling to larger columns should be possible and the relatively expensive ion-pair reagents are not longer necessary. The mobile phases without ion-pair reagents allow the direct coupling of the HPLC to a mass spectrometer.

A NEW FLUORIMETRIC HPLC METHOD FOR THE DETERMINATION OF ACIDIC POLYETHER TOXINS IN MARINE PHYTOPLANKTON

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Phytoplankton samples were harvested off the south-west coast of Ireland using a large composite plankton net (590 x 120 cm) with an outer net of 50 μm and an inner net of 108 μm mesh sizes which was effective for collecting biomass rich in Dinophysis sp. A new rapid, sensitive HPLC method has been developed for the determination of acidic diarrhetic shellfish poisoning (DSP) toxins and this has been applied to the determination of toxin profiles in phytoplankton. Extracts from phytoplankton samples were reacted with 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1)-quinoxalinone (BrDMEQ) and 5% diisopropylethylamine at 50C for 20 min. Sample cleanup was achieved using silica solid phase extraction (SPE) with a 83 +/-2% recovery for okadaic acid (OA) derivatives. Isocratic reversed phase HPLC was carried out using acetonitrile/water (57/43) with fluorimetric detection. Calibrations were linear (R2 = 0.991) in the typical analytical range of 1 – 10 ng OA equivalents injected. OA, DTX-2 and pectenotoxin-2 seco acids (PTX2SA) were determined in phytoplankton as their DMEQ derivatives and toxin identification was confirmed using LC-MS and LC-MSn. In a comparative study with the reagent, 9-anthryldiazomethane (ADAM), which was applied to the analysis of toxins in phytoplankton, the BrDMEQ method was not only more sensitive than the ADAM method but improved resolution of the polyether toxins derivatives was achieved. Although, both OA and DTX-2 were found in all phytoplankton samples that contained *Dinophysis* sp., PTX2SA compounds were detected in only a small number of samples.

THE IMPORTANCE OF AKINETES IN THE BLOOM DYNAMICS OF TOXIC CYANOBACTERIA -STUDIES ON *ANABAENA CIRCINALIS* FROM AUSTRALIAN WATERS.

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Paralytic shellfish toxin producing blooms of *Anabaena circinalis* are a frequent occurrence in freshwaters throughout Australia, spanning tropical to cool temperate regions. These blooms pose serious management and health issues, with blooms sometimes extending for over 1000 km and with stock deaths associated with blooms. We are investigating the role of akinetes in the bloom dynamics of *A. circinalis*. Akinetes are presumed to be a resistant resting stage due to their capacity to survive environmental conditions which are deleterious to vegetative cells and because of their ability to form germling cells. We are using cultured strains isolated from blooms representing the diversity of biogeographic regions in Australia. We found some evidence of akinete production in response to limiting phosphorus but the effect was not consistent for all strains tested. Low temperature, temperature shock treatment, anaerobia, low salinity stress, and light intensity extremes had no effect on akinete production. Differentiation appeared to be actively inhibited by blue and short duration UV light but significantly promoted by red and white light rich in red wavelengths. Similarly to the results for differentiation, the optimal temperatures for germination matched those for vegetative growth. Akinetes remained viable but didn't germinate at the lower temperature extremes (5^0C) and were killed at high temperatures (40^0C). Both the light quality history during differentiation and the actual germination light quality had no influence on germination frequency but germling growth was favoured by white light enriched in red wavelengths. We observed germination after 24 hours under anaerobic/microaerobic conditions but this was followed by zero survival. Germling cell growth rates were greater than those measured for vegetative cells across a range of germination experiments. The implications for these results on bloom dynamics will be discussed.

UV-ABSORBING COMPOUNDS IN MARINE MICROALGAE: FOCUS ON BLOOM-FORMING SPECIES

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Harmful algal blooms present a growing problem for fisheries, coastal ecosystems, aquaculture and public health. A widespread belief suggests that these outbreaks are due to increasing coastal eutrophication. Other factors, such as global climate change (including ozone depletion and increasing UV radiation) may also affect algal blooms and successions by differential tolerance of species to environmental stress. We examined 152 species (206 strains) of cultured microalgae from 12 classes for the presence of UVA- and UVB-absorbing compounds. Cultures were grown under white fluorescent light with no supplementary UVA or UVB radiation. Tetrahydrofuran/methanol extracts of microalgae were examined for ratios of UV absorbance (280-390 nm) to chlorophyll a (665 nm). Three groups of species were found: those with low UV : chl a ratios (0.18 to 0.9, diatoms, green algae, cyanophytes, euglenophytes, eustigmatophytes, rhodophytes, some dinoflagellates, some prymnesiophytes), those with intermediate ratios (0.9 to 1.4, chrysophytes, some prasinophytes, some prymnesiophytes), and those with very high values (1.4 to 6.75, surface bloom-forming species of dinoflagellates, cryptomonads, prymnesiophytes and raphidophytes). HPLC analysis of bloom-forming raphidophytes and dinoflagellates showed suites of UV-absorbing mycosporine-like amino acids including mycosporine-glycine, asterina-330, shinorine, porphyra-334 and palythine. Local strains of the successful toxic bloom-forming dinoflagellate, *Gymnodinium catenatum*, also contained major quantities of unknown UV-absorbing compounds. Evidence suggests that the presence of UV 'suncreens' may allow bloom-forming species to dominate in UV-rich environments.

GROWTH AND GRAZING RATES OF THE HETEROTROPHIC DINOFLAGELLATE *OXYRRHIS MARINA* ON A TOXIC DINOFLAGELLATE *AMPHIDINIUM CARTERAE*

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We investigated growth and grazing rates of the heterotrophic dinoflagellate *Oxyrrhis marina* on a toxic dinoflagellate *Amphidinium carterae. O. marina* grew well on *A. carterae*. Specific growth rates *of O.marina* increased rapidly with increasing prey density up to ca. 200 ng Cml-1, but were saturated at higher concentrations. Maximum specific growth rate of *O. marina* on *A. carterae* was 0.8 day-1. Maximum ingestion and clearance rates of *O. marina* were 5 ng C grazer-1 day-1 and 1.2 nl grazer-1 h-1, respectively. *O. marina* exhibited much higher maximum growthrate than the heterotrophic dinoflagellate *Polykrikos kofoidii* on the sameprey species. *O. marina* might have ability of detoxicating toxins andconsiderable grazing impact on *A. carterae.*

SPIROLIDE PRODUCTION AND PHOTOPERIOD-DEPENDENT GROWTH OF THE MARINE DINOFLAGELLATE *ALEXANDRIUM OSTENFELDII*

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The gonyaulacoid dinoflagellate *Alexandrium ostenfeldii* (Paulsen) Balech et Tangen has recently been identified as the source of spirolides,pharmacologically active macrocyclic imines in natural plankton populations and shellfish. The effects of physiological status onspirolide production were studied by comparing nutrient-replete growthof a toxic strain of *A. ostenfeldii* (SH98) isolated from Nova Scotiawith that of a non-toxic clone (BAHME 146) after dark-induced cellsynchronization. Clones were grown in triplicate 15 L batch cultures at 15C under a 14:10 L/D photocycle at an ambient photon flux densityof 260 μmol m-2 s-1. After 106 h dark adaptation, samples were taken at2 h intervals through three L/D cycles for measurement of chlorophyll a(extracted and in vivo), cell number, cell size, cellular DNA, andspirolide concentration. Although complete cell synchronization was notachieved, cell size variation was related to the L/D photocycle, withsize increasing in the light and decreasing in the dark. Mean cellnumbers decreased during the dark, but net growth was positive duringthe experiment (μ =0.18 div d-1). The extracted chlorophyll a exhibitedthe same trend as the cell concentration, with no apparent shift in theamount of chlorophyll a per cell in relation to the L/D phase. Analysisby liquid chromatography-mass spectrometry (LC-MS) showed that thespirolide profile did not vary significantly, consisting primarily ofthe des-methyl-C derivative (>90% molar), with analogues C, C3, D, D3and des-methyl-D as minor constituents. For the toxigenic clone, thetotal spirolide concentration per unit culture volume was directlyrelated to the concentration of cells and chlorophyll a, but there was adramatic increase in cell quota of spirolides at the beginning of thedark phase and a corresponding decrease in the light. Biosynthesis ofthese polyketide-derived metabolites is apparently affected bylight-dependent events in the cell cycle.

THE USE OF A DIVING-PAM FOR DETECTION OF IRRADIANCE, BIOMASS AND PHOTOSYSTEM II FLUORESCENCE YIELDS IN HARMFUL ALGAL BLOOM EXPERIMENTS: DISCUSSION OF METHODOLOGICAL ASPECTS.

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The use of Pulse Amplitude Modulated fluorometer (PAM) has in the last tenyears been widely used in higher plant photo-physiology. In marine biology thePAM technique has basically been used the last six years on culture work onphytoplankton. Since the introduction of the DIVING-PAM in 1996, it hastypically been used for fluorescence yield measurements from macroalgae andcorals containing zooxanthellae (algal symbionts). We report the use of thisinstrument in harmful phytoplankton bloom research. Optically thick layers of toxic dinoflagellates such as *Alexandrium tamarense* grown in cultures flaskswith different nitrate : phosphorus ratios, have been used for directfluorescence yield measurements (operation quantum yield for stable chargeseparation at photosystem (PS) II, IIe). We also show examples of the use ofthis instrument during mesocosm experiments. The use of IIe in combination withabsorbed quanta, and the corresponding fraction of absorbed quanta directed toPS II gives information of pigment-group present and photo-physiological state.Oxygenic photosynthesis from PS II can be estimated based on optical andbio-optical measurements, i.e. the combined information of irradiance (400-700nm), spectral irradiance, temperature, IIe, Chl a-specific absorptioncoefficients (400-700 nm) and scaled fluorescence excitation spectra (400-700nm) to estimate mg O2 produced mg Chl a-1 h-1. This bio-optical information maybe the future tools for in situ monitoring of harmful algal blooms and as anaid to estimate photosynthetic performance and growth rates of phytoplankton.

INVESTIGATIONS INTO THE ROLE OF PLASMIDS IN THE METABOLISM OF PARALYTIC SHELLFISH TOXINS (PSTS) BY MARINE BACTERIA

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Recent research has demonstrated that a variety of bacteria, isolated from bivalve molluscs, have the ability to metabolise paralytic shellfish toxins (PSTs). As such it has been suggested that marine bacteria may play a role in the clearance of PSTs from shellfish species. The mechanisms by which bacteria metabolise these toxins isunknown. However, there is existing evidence from some bacterial strains that the metabolism of compounds, such as lactose and glucose, can be plasmid-mediated and it is feasible the genes involved in bacterial metabolism of PSTs may be carried on bacterial plasmids. In this study, bacterial isolates able to metabolise a range of PSTs (GTX º, GTX 2/3, STX, neoSTX, C1 and B1) have been examined for plasmid content with a view to determining their role in toxin conversion. Results will be presented to illustrate the effect of plasmid curing on the PST metabolising capabilities of the derivative strains.

HARMFUL ALGAE BLOOMS AND THE UPWELLING REGIME IN ATLANTIC COASTAL WATERS OF MOROCCO

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The Atlantic shores of Morocco extend about 3000 Km from the Straits of Gibraltar to Cap Blanc (21^0 N), and are bathed by the Canary Current. This is one of the major coastal upwelling regions of the world. Upwelling occurs throughout the year and is most intense in spring and summer. Algal blooms often affect the area from Cape Juby to Gibraltar (28^0 N to 36^0 N) where upwelling is more active in summer.

The first records of red tides in Atlantic Moroccan waters date from 1966. Since then, red tides have been recorded regularly during late summer and early autumn. The years 1971, 1975 and 1982 were marked by shellfish contamination with PSP levels exceeding the public health safety threshold and their consumption was forbidden. The species responsible were not identified. In October-November 1994, an exceptional bloom of *Gymnodinium catenatum* appeared on the coast between 31 N and 35 N and caused a human poisoning near Casablanca (33; 39, N). In August 1998 extensive red tides occured in coastal waters between 28 N and 33 N. *Alexandrium minutum, Prorocentrum micans, Scrippsiella* sp and *Lingulodinium polyedrum* were the main species. In July and August 1999 a progressive monospecific bloom of *Lingulodinium polyedrum* occured in the same area progressing from North to South, and caused DSP contamination in shellfish tested by mouse bioassay. The seasonal variability of upwelling on the Moroccan coast between 1994 and 1999 has been investigated and compared with the occurrence and timing of harmful algae blooms. The results of this study for the continental shelf from Casablanca (33; 30' N) to Cape Blanc (21N) will be discussed.

A NEW RED TIDE-FORMING SPECIES *PERIDINIUM QUINQUECORNE* ABE IN SOUTH CHINA SEA

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A bloom of the dinoflagellate *Peridinium quinquecorne* Abe firstly occurredin a eutrophic shallow waters in South China Sea and the waters discolored.The morphology and structure of the organism were studied through LM andSEM. The highest population density ($11.8.10^6$ cells.L-1) was counted inintensively stained waters (Ya-qian Bay). The bloom lasted for more than 10days and disappeared when the temperature turned down sharply. Nutrient analyses with samples taken from different stations showed the species preferring eutrophic environments. Unlike many other red tide species of dinoflagellate, *P. quinquecorne* seems not to need a regular N:P ration for growth. Some factors related to bloom occurrence were discussed.

THE EFFECT OF FLUID FLOW ON THE CELLULAR TOXIN CONTENT OF *ALEXANDRIUM FUNDYENSE*

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Net population growth of many red-tide dinoflagellates is reduced by exposure to fluid flow.In previous experiments using the red-tide species *Lingulodinium polyedrum*, net population growth was reduced by as much as 50% by exposure to laminar shear stress of 0.004 N m^{-2} for 1-4 h d^{-1}. Flow cytometric cell cycle analysis demonstrated that reduced population growth was due to reduced division rate regardless of exposure duration. The reduction in division rate appeared to be due to a lengthening of the G1 phase of the cell cycle.

A similar lengthening of the G1 phase probably explains the flow-induced reduction of population growth of dinoflagellates in the closely related genus *Alexandrium.* Others have shown that the saxitoxin-producing dinoflagellate *Alexandrium fundyense* produces toxin only in the G1 phase. Therefore, extending the G1 phase by exposing *A. fundyense* to shear is hypothesized to increase cellular toxin content. In the present study, *A. fundyense* cultures grown on a 12:12 h LD cycle were exposed to constant laminar shear generated in Couette flow chambers for 1, 4 or 24 h d^{-1} for 5-8 days. Shear stress during the daily exposure period was 0.004 or 0.01 N m^{-2}. Saxitoxin equivalents were measured with a receptor binding assay using rat brain synaptosome membrane fractions. Preliminary results demonstrate that population growth of *A. fundyense* is inhibited by shear and cellular toxin content is correlated to the level of growth inhibition. Extrapolating these results to field conditions, oceanic turbulence sufficient to inhibit the growth of toxic red-tide dinoflagellates may also increase cellular toxin content.

PHYSICAL AND CHEMICAL FORCING OF ALGAL BLOOMS IN DANISH MARINE WATERS

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The occurrences of harmful blooms in Danish coastal waters during the past20 years have been analysed. Harmful algal blooms are a recurrent phenomenonin Danish marine waters. The blooms develop in the saline waters off thewestern coast of Jutland (=eastern North Sea and Skagerrak) and in the"inner coastal water" (Kattegat and Belt Sea = brackish waters between theNorth Sea and Baltic Sea). The harmful blooms may be divided into two groups: the regular blooms and the exceptional blooms. Regular blooming species are *Prorocentrum minimum,Gymnodinium mikimotoi*, and *Nodularia spumigena*. Among the most well known exceptional blooms are the *Chrysochromulina polylepis* bloom in 1988 and the *Chattonella* sp. blooms in 1998. The harmful effects of the blooms vary fromnot detectable (*Prorocentrum minimum*) to mass death of bottom fauna and other fauna/flora (*Gymnodinium, Chrysochromulina*). Common for blooms is that they are often followed by oxygen depletion. In general the frequency of blooms has not increased (or decreased).To improve the forecast of blooms a number of large scaled blooms have beenanalysed. Biological, chemical and hydrographic data on the blooms are collected by the Danish counties and the National Environmental Research Institute. In addition, meteorological data and satellite image are used forthe analyses. Using case stories the most important factors inducing bloomsin Danish marine waters will be discussed. Not surprisingly, the decisive factors that can be use in prognostication are weather conditions, i.e. wind, irradiance, precipitation and derivative parameters as mixing, currents, photon fluxes and nutrients. Danish marine waters are however complex due to the location in the transition zonebetween the very brackish Baltic Sea and the marine North Sea. Comprehensive knowledge of cause-effect relations is thus needed to make operationalmodels that can predict blooms. The potential of making a prognostic model for the Danish marine waters is demonstrated.

REAL-TIME MONITORING FOR TOXICITY CAUSED BY HARMFUL ALGAL BLOOMS AND OTHER WATER QUALITY PERTURBATIONS

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Harmful algal blooms (HABs), including those associated with toxicity,have increased in frequency and severity in U.S. coastal areas andworldwide. Recently, the U.S. mid-Atlantic region experienced blooms of*Pfiesteria* and *Pfiesteria*-like organisms that were associated with fishkills that affected local fisheries, recreation and tourism, and evoked serious concerns about human health. Unfortunately, environmental managers responsible for closure of waterways cannot determine if a fishkill event is HAB-related until large numbers of dead or moribund fish are observed and there is confirmation of the presence of HAB species. To address this problem, an automated biomonitoring system has been developed to continuously monitor waters that are susceptible to HABs and otherwater quality perturbations. The system consists of a series of chambers to expose sentinel fish to flow-through water. Paired electrodes in eachchamber non-invasively transmit an amplified electrical signal from eachfish to an on-site computer. A water quality module records and transmitsdata on dissolved oxygen, temperature, conductivity and pH. Data can betransmitted to remote locations for further analysis and response.Computer software analyzes input voltages and converts them to real-timeventilatory rate readouts. Other physiological measurements from the same voltage signal may also be discerned by software algorithms that arecurrently under development. Preliminary laboratory trials indicate that sublethal hypoxia and exposure to toxins produce consistent alterations infish ventilatory response in real-time. Studies are underway to discernresponse differences for different stressors as well as remote fieldapplication of the biomonitoring system. System design, experimental dataand overall utility of the system for HAB biomonitoring will be presented.This study has been supported in part by a U.S. Environmental ProtectionAgency EMPACT grant, #DW21938658-01-1.

FISH LESIONS IN THE CHESAPEAKE BAY: *PFIESTERIA*-LIKE DINOFLAGELLATES AND OTHER ETIOLOGIES

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Ulcerative lesions and mass mortalities of Atlantic estuarine fish,particularly menhaden (*Brevoortia tyrannus*), have been associated withexposure to *Pfiesteria*-like dinoflagellates and their toxins. We will discuss the pathology of fish collected from the Chicamacomico River,Maryland. In the majority of menhaden sampled we observed solitary ulcerative lesions on the trunk or around the vent. One striped bass(Morone saxatilis) had an area of reddening around the base of the dorsalfin. Bluegill (*Lepomis macrochirus*), channel catfish (*Ictaluruspunctatus*), yellow perch (*Perca flavescens*) and carp (*Cyprinuscarpio*)were externally non-remarkable. Histologically, ulcerative menhaden lesions demonstrated a marked chronic inflammatory infiltrate in largeareas of exposed necrotic muscle. The ulcers contained granulomata with fungal hyphae in the necrotic tissue. Gram negative rod-shaped bacteriawere also observed in the lesions, a common finding in ulcers of aquaticorganisms. Our data suggest that "typical" ulcerative lesions observed onfish from areas of *Pfiesteria*-like dinoflagellate blooms are reflective of dermatosis which may be related to a variety of individual or combined environmental stressors. Exposure to dinoflagellate toxin(s) potentially represents one such stressor, and the role of *Pfiesteria*-like dinoflagellate toxin in fish primary lesion development is currently under investigation. We will also discuss the presentation of different ulcerative lesions observed in other Chesapeake Bay fish pathology studies. These studies have been supported in part by the U.S. Army Garrison Aberdeen Proving Ground, Installation Restoration Program, and agrant from the U.S. Environmental Protection Agency #CR826913-01-0.

ELEMENTAL COMPOSITION OF C, N AND P IN SINGLE FILAMENTOUSCELLS OF MARINE CYANOBACTERIA USING NMP (NUCLEAR MICROPROBE)- AND STANDARD METHODS

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There is a need to find a technique for analysing the intracellular concentra-tions of carbon, nitrogen and phosphorus in single phytoplanktoncells growing in natural communities. Laboratory experiments with uni-algal cultures have shown that the carbon (C): nitrogen (N): phosphorus (P)ratios under balanced nutrients conditions is species-specific and do notalways follow the Redfield ratio. In this study we apply a method using aNMP-technique to measure the concentration of C, N and P in single cells ofthe three brackish-water cyano-bacteria species *Anabaena* sp., *Nodulariaspumigena* and *Aphanizomenon* cf *klebahnii.* The concentrations of C-, N- andP were also analysed by conventional methods and comparison was made withthe results obtained from the NMP-technique. *Anabaena* sp. showed no significant difference regarding the C-, N- and P- concentration with the two methods. In the case of *N. spumigena* and *A. cf klebahnii* the C, N and P concentrations showed approximately a 50% higher and a 50% lower concentration respectively with the NMP technique than theconvential methods. Except for the N/P ratio for *A. klebahnii,* the C/N-,N/P-, and C/P ratios for all three species showed no significant differencebetween the two techniques. From our results it can be concluded that theuse of NMP could be a useful tool for studying the elemental composition insingle phytoplankton species in the field.

COMPARISON STUDIES ON THE TOXICOLOGY OF TWO JAPANESE STRAINS OF *HETEROSIGMA AKASHIWO* (RAPHIDOPHYCEAE)

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Toxin profiles and ichthyotoxicity of two strains of *Heterosigma akashiwo* (SI78 and KB95) grown under the same laboratory conditions were compared at different growth phases. The strains SI78 and KB95 were collected from Seto Inland Sea and Kagoshima Bay, Japan, during the massive red tide outbreaks in 1978 and 1995. There were great variations in the toxin profiles between two strains, and the study of toxin profiles indicated that the strain SI78is several times more toxic than the strain KB95. The strain SI78 contained a large proportion of HaTx-I (corresponding to PbTx-2) with lesser amounts of HaTx-IIa (corresponding to PbTx-9), HaTx-IIb (corresponding to PbTx-3)and HaTx-III (corresponding to oxidized PbTx-2) while KB95 contained mainlyHaTx-III with small amounts of HaTx-I and HaTx-IIb. HaTx-IIa was notdetectable in the strain KB95 but a very small amount in mid logarithmic phase. In ichthyotoxicity studies, SI78 showed less growth, but was more toxic than KB95. SI78 exhibited its highest toxicity on the 6th day (0.018FU) at a cell density of 41,475 cells/ml, whereas in KB95 the highest toxicity was found on the 8th day (0.004 FU) at a cell density ofapproximately 142,300 cells/ml. During the growth cycle, there was a significant fluctuation in the concentration of all toxic fractions. In both isolates, the amounts of HaTx-I and HaTx-IIb were relatively low after inoculation, increased rapidly in logarithmic phase, and then decreased asthe culture aged. The yield of HaTx-III was low in early to mid logarithmic phase and started to increase in late logarithmic phase.

MICROCYSTIN-AW, A NEW MICROCYSTIN VARIANT ISOLATED FROM CYANOBACTERIAL WATERBLOOMS IN THAILAND

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Recently, freshwaters in Thailand have been eutrophicated by industrial and municipal wastewater. Toxic cyanobacterial waterblooms have occurredfrequently in the freshwaters. The major toxin in the waterblooms has beenidentified as microcystin-LR and -RR and their 6(Z) forms. Also other microcystin variants such as microcystin-YR, -LA, -ThtyrR and -AR were detected as minor toxins. During investigation of microcystin incyanobacterial waterblooms in Thailand, we found a novel microcystin variant. The waterbloom sample was collected from a pond in Lumpoon, and was consist of *Microcystis aeruginosa*, Microcystin was extracted with methanol, and was fractionated using Sep-Pak ODS cartridges. Each of microcystin variants were separated by HPLC using a reverse-phase column,and purified by silicagel TLC using chloroform / methanol / water (60/40/1,v/v) as the solvent. The chemical structures of the variants were analyzed using two-dimensional NMR and HRFABMS. The waterbloom contained microcystin-RR, -YR, -LR and -WR as known variants, and a hydrophobic unknown variant. From the results of the analyses and determination ofamino acid configuration, the unknown microcystin variant was identified as microcystin-AW.

OCCURRENCE OF PROROCENTRUM LIMA IN COASTAL WATERS OF THE GULF OF MAINE, USA

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Prorocentrum lima (Ehrenberg) Dodge has been found at several sites along the coast of Maine during the last two summers, some in areas where shellfish are harvested commercially. Identity was confirmed by SEM. This species was first observed in the Gulf of Maine in 1994 in an offshore plankton net sample, but has not been previously reported from coastal locations. Some samples containing the dinoflagellate originated from aquaculture sites, while others were associated with wild mussels collected at low tide. Many of the cells were isolated from water samples and net tows, and were not found in association with epiphytic algae. The sites were extremely shallow, so resuspension of benthic cells may account for their presence in the water column. In other cases, cells were collected from material rinsed off clumps of the blue mussel, Mytilus edulis. Although this dinoflagellate is known to produce toxins (okadaic acid and derivative compounds), no incidence of diarrhetic shellfish poisoning (DSP) has been documented so far in the Gulf of Maine, despite toxicity events in the early 1990s in Nova Scotia, Canada, immediately to the north. Although Prorocentrum lima appears to be relatively rare in Maine coastal waters, its apparent widespread distribution warrants increased monitoring to allay public health concerns.

HARMFUL ALGAL BLOOMS IN THE WETLANDS OF THE SWAN COASTAL PLAIN, WESTERN AUSTRALIA

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Wetlands in the Swan coastal plain are typically shallow and alkaline withincreasing electrical conductivity and nutrient enrichment. More than 50 % of the lakes can be classified as mesotrophic and eutrophic. Algal blooms are common in the Spring, Summer and Autumn period in eutrophic and hypertrophic wetlands. Surveys conducted in 1993, 1998 and 1999 indicated cyanobacterial blooms following green algal blooms during the Spring and Summer seasons.

This paper presents an overview of the succession and seasonal pattern ofalgal blooms in the wetlands of the Perth Metropolitan area. The southernwetlands, Bibra Lake, North Lake, Forrestdale Lake, all of which can be described as hypertrophic, experience blooms of *Microcystis aeruginosa,Anabaena spiroides, A. circinalis* and occasionally *Nodularia spumigena.*In 1993, Forrestdale Lake displayed blooms of all the above species. The physio-chemical conditions with the blooms have been investigated. High conductivity (>500 μs/cm), high pH and low N:P ratio were associated with both *Anabaena* and *Nodularia*; lakes dominated by *Microcystis aeruginosa* hada higher N:P ratio.

Canning River – a tributary of the Swan River estuary – has experiencedsevere blooms of *Anabaena spiroides* and *A. circinalis* in recent years. Introphic status and physio-chemical properties, this river is similar to the southern hypertrophic wetlands. Of all the blooms in the freshwater wetlands, *Microcystis aeruginosa* blooms are the most common. Currently, we are involved in collecting data on theecology and distribution pattern of *Microcystis aeruginosa*, along with anytoxins produced by this species. Our objectives are to record thedifferent morphological forms of the cyanobacterium and relate their distribution to environmental conditions and to investigate any possible connections between morphology and toxicity.

BREVETOXINS INDUCE EMBRYO TOXICITY AND DEVELOPMENTAL ABNORMALITIES

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Brevetoxins are lipophilic polyether toxins with documented neurotoxic effects on adult animals. In this study, we extend an earlier study ofciguatoxin (Toxicon 37:1827,1999) to quantify the adverse developmental effects of brevetoxins using an exposure paradigm that parallels the maternal-oocyte transfer of toxin. Medakafish (*Oryzias latipis*) embryos areexposed to brevetoxin six hours post fertilization by microinjection of asmall quantity (2 nanoliters) of brevetoxin (or vehicle) reconstituted in afish oil (triolein) droplet. The brevetoxin-containing droplet is placed adjacent to the larger oil droplet of the yolk sac. Embryos microinjected with doses of 0.8 ng/egg (ppm) and higher of brevetoxin-1 exhibit pronouncedcardiovascular (tachycardia) and muscular (hyperkinesis) activity by embryonic day four. Prior to hatching, morphological abnormalities were commonly found in embryos at the following lowest adverse effect levels: 1.1ppm- lateral curvature of the spinal column; 3.1 ppm- herniation of brainand meninges though defects in the skull; and 3.4 ppm malpositioned eye.Hatching abnormalities are also commonly observed at brevetoxin doses of 2.0ppm and higher with head-first, as opposed to the normal tail-firsthatching. Given the similarity of developmental processes found betweenhigher and lower vertebrates, teratogenic effects of brevetoxins have the potential to occur among different phylogenetic classes. The observation of developmental abnormalities following brevetoxin exposure identifies a new spectrum of adverse effects that may be expected to occur following exposure to red tide events.

A PILOT STUDY TO EXPLORE THE RELATIONSHIP OF OCCUPATIONAL EXPOSURE TO *GYMNODINIUM BREVE* TOXIN AND PULMONARY FUNCTION

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The Western Coast of Florida frequently experiences a harmful algalbloom caused by the dinoflagellate, *Gymnodinium breve (G. Breve). G.Breve* releases a toxin when the cells lyse, and this toxin becomes part of the marine aerosol. When humans are exposed to *G. Breve* toxin inmarine aerosol, upper respiratory symptoms such as runny nose, nasal congestion, cough, and sore throat are commonly reported. Since the estimated size of the particle is 7 – 10 μm most toxin should be filtered by the upper airway. If the lower airway is impacted by the red tide toxin, bronchoconstriction of the smooth muscle may occur. The common method to detect bronchoconstriction is the measurement of the Forced Vital Capacity (FVC) and the forced vital capacity exhaled in 1 second (FeV1). The purpose of this pilot study was to see if occupational exposure to red tide toxin during a scientific research cruise could be detected through spirometry. A spirometer providing hard copy of the flow volume loop was placed on three research cruises in 1998 and 1999. Data on the third research cruise is currently being collected. The primary purpose of these cruises was to conduct experiments in a red tide bloom in the Gulf of Mexico. Fifteen volunteer scientists were instructed on the correct method to perform the FVC maneuver. They were also asked to complete a Health History Questionnaire (Hollister Inc, 1980). The volunteers then performed a forced vital capacity maneuver at varying times of the cruises and also documented any respiratory symptoms. Variation in FVC and FeV1 in correlation to cell counts and aerosol collection will be discussed. This is an initial investigation of the occupational exposure to the red tide toxin in the marine aerosol.

PIGMENT COMPLEMENT SUPPORT FOR THE ASSIGNMENT OF A NEW GENUS WITHIN GYMNODINIALES

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Evidence from HPLC pigment analyses supports the assignment of several dinoflagellate species to a new genus. The carotenoid gyroxanthin-diester has previously been reported in several species of dinoflagellates including *Gymnodinium breve* Davis, *G. galatheanum* Braarud and *Gyrodinium aureolum* Hulbert. In the study reported here, four other dinoflagellate species have been found to contain gyroxanthin-diester as well as chlorophyll c1/c2, chlorophyll c3 and diadinoxanthin. The fucoxanthin and 19'-acylofucoxanthin complements show species-specific differences, varying widely from near equal quantities of each to exclusively 19'-acylofucoxanthin. Two isolates of *Gymnodinium mikimotoi*, one from New Zealand and one from Japan, had nearly indistinguishable pigment complements. This group of dinoflagellates identified as being substantially dissimilar from other genera to warrant a new genus do not contain peridinin. These results from the pigment analyses will be presented in conjunction with other presentations which will discuss the molecular, morphological, ultrastructural, biochemical and toxin data that support the suggestion of a new genus.

DEEP CHLOROPHYLL MAXIMUM CREATED BY *HETEROCAPSA TRIQUETRA* EHRENBERG AT THE ENTRANCE TO THE GULF OF FINLAND, BALTIC SEA

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Development of a deep chlorophyll maximum layer (DCM) formed by *Heterocapsa triquetra* Ehrenberg was studied during a 3-week multidisciplinary research cruise at the entrance to the Gulf of Finland in July 1998. The fluorescence mappings made with the towed undulating device carrying CTD+fluorometer revealed the existence of the DCM in the lower part of the chlorophyll containing layer at the depth of 20-40 m. The DCM appeared mainly as patches of 1 km horizontal scale. The thickness of DCM varied between 0.2 and 5 m . The Chl *a* concentration in samples taken by a decimeter-scale sampler from the DCM extended up to 9 mg m-3, being comparable with the concentration in the surface layer. According to the microscopical examination the DCM layer was formed solely by the dinoflagellate species *H. triquetra* and there was a good correlation between Chl *a* and the abundance of *H. triquetra.*The population was viable and started to phosynthesize as soon as exposed to surface illumination conditions. In some cases the vertical positioning of the DCM was found to be correlated with the pecularities in thermohaline structure, but this was not a rule. In every case the DCM coincided with the sharpest part of the nitracline. As an explanation for the nitracline development, the roles of nitrate uptake and physically mediated vertical transport of nitrate were estimated on the basis of measurements of cell number, nitrate concentration and dissipation rate.

THE *ALEXANDRIUM* GENUS IN THE FAR EASTERN SEAS OF RUSSIA AND ADJACENT WATERS OF PACIFIC OCEAN

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In the Far Eastern seas of Russia 9 species of *Alexandrium*, including one dubious species *A.* (?) *Gonyaulax lebourae* Balech, were found. Three species are new for the Far Eastern seas and the seas of Russia. They were found during summer-autumn period; population densities were relatively high: 10^5 cell/L for *A. acatenella* (Whed.et Kof.) Balech (Avachinskaya Guba Inlet, Pacific coast of Kamchatka), 10^4 cell/L for *A. insuetum* Balech (Peter the Great Bay, Sea of Japan) and 10^3 cell/L for *A. pseudogoniaulax* (Biecheler) Horiguchi, ex Yuki & Fukuyo (Peter the Great Bay and Aniva Bay, Sea of Okchotsk). The morphology of these species is described. *A. ostenfeldii* (Pauls.) Balech et Tangen and *A. tamarense* (Lebour) Balech occur in all Far Eastern of Russia, the former species always shows a low concentration. *A. tamarense* forms red tides, sometimes toxic in the Bering sea and along the Pacific coast of Kamchatka; maximum density is up to $2x10^6$ cell/L. A primitive life cycle of *A. tamarense* from Avachinskaya Guba Inlet (Kamchatka) is described.

CONFIRMATION OF DOMOIC ACID PRODUCTION BY *NITZSCHIA* SP. NOV. (BACILLARIOPHYCEAE) CULTURES, ISOLATED FROM VIETNAM IN 1997 AND 1998

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Domoic acid (DA), a neurotoxic amino acid, was detected by HPLC-fluorescence analysis in 5 monoclonal cultures of *Nitzschia* sp. nov. (Bacillariophyceae), isolated from a shrimp culture-pond at Do Son, Vietnam in 1997. DA levels of the cultures ranged from 1.2 to 3.1 (average 2.3) pg/cell, which was within the amounts reported for *Pseudo-nitzschia multiseries.* Confirmation of DA was performed by ESI MS after purification of the DA-like substance which was extracted from the cells of 3 L culture of one strain. DA production of the new species were investigated by batch culture experiments under axenic and non-axenic conditions. Like in many *Pseudo-nitzschia* species, DA production of both cultures started from late exponential growth phase and reached a maximum at early stationary growth phase followed by gradual decrease. In contrast to *P. multiseries*, no significant difference was observed in DA production between axenic and non-axenic cultures. Five monoclonal cultures of the same species were also established from Ha Long Bay, Vietnam in 1998. DA production of them were also confirmed by HPLC analysis.

BIOLOGICAL AND ECOLOGICAL CHARACTERISTICS OF *GYMNODINIUM CATENATUM* IN JAPAN

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Based on previous papers and results from recent investigations, we present the biological and ecological characteristics of Japanese strains of *Gymnodinium catenatum*. Distribution, oceanographic conditions for blooming and the toxin composition of this toxic dinoflagellate in Japan including cysts in the sediment will be detailed. Recently, *G. catenatum* blooms have been increasing in the coastal waters of western Japan, e.g. Kyusyu and western Shikoku. During the bloom periods, wild oyster, short-necked clam and cultured noble scallop have been contaminated by PSP. Before 1996, the PSP events by *G. catenatum* had been restricted to Senzaki Bay, Japan Sea coast of western Honshu, where PSP by this species was first confirmed in 1986. Throughout the study sites in the coastal waters of western Japan, *G. catenatum* blooms have been observed in enclosed bay areas over a wide range of water temperatures, 6-27 degrees centigrade, and under different seasons. Major toxin components of C1+C2, GTX6 and GTX5 in the strains we examined are the same in other Japanese strains so far examined. Cysts in sediments were found throughout the study sites, although the cyst densities were low levels with below 10 cysts/g of wet sediment. Therefore, expansion of *G. catenatum* distribution in western Japanese waters shall be expected with fears of further PSP outbreaks.

ABSORPTION SPECTRA OF *NODULARIA SPUMIGENA* AND *APHANIZOMENON FLOS-AQUAE* REVEAL CHROMATIC ADAPTATION TO THE LIGHT FIELD IN THE BALTIC SEA

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Every summer, *Nodularia spumigena* and *Aphanizomenon flos-aquae* produce big blooms in the Baltic Sea. These blooms are potentially toxic and have attracted a lot of public interest in recent years. The two species are so competitive because they are diazotrophic, i.e. they can fix elemental nitrogen, and are very well adapted to the low DIN:DIP ratio in the Baltic Proper during summer. Absorption spectra of the two cyanobacteria species were measured for samples from laboratory cultures grown under white light, as well as from field samples from the Baltic Sea. The laboratory samples of both species showed a distinct absorption peak in the light-red (630-640 nm). The spectra from the field samples do not show any distinct feature in that area of the spectrum, but have a shoulder in the green part of the spectrum (at about 570 nm). Absorption measurements of all optical in-water constituents show a distinct trough in the same part of the spectrum, and therefore imply chromatic adaptation of both species to the light field in the Baltic Sea. The paper examines the distinct absorption spectra of the two species and tries to examine the possibilities of monitoring these species by remote sensing.

THE USE OF A SAILING SHIP FOR MONITORING TOXIC ALGAL BLOOMS IN THE BALTIC SEA

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Searcher is a small Swedish research vessel for expeditions to islands which are difficult to reach, but was used last summer (end of August/beginning of July) for a project that investigates the possibilities of using satellite remote sensing for monitoring toxic algal blooms in the Baltic Sea. This project consists of a collaboration between Stockholm University and the University of Maryland. The blooms are mostly formed by two species, *Nodularia spumigena* and *Aphanizomenon flos-aquae* with *N. spumigena* being the predominant organism. Because of the good spatial coverage, remote sensing provides an excellent tool for monitoring the movements as well as the spatial coverage of the bloom. Satellite data was automatically processed near-real time by the SeaWiFS project and sent to our ground station in Gotland. This information, combined with the information from the Swedish Coast Guards, helped us to find adequate positions for our ground truth stations. The optical in-water constituents were measured in-situ, as well as in the lab, and Searcher proved to be much more appropriate for this kind of work than a big research vessel – the main advantage being that a bloom patch could be approached carefully without severe disturbance of the water column and a turnover of the stratified water body. Information about Searcher, and about this cruise in particular can be found on: http://www. searcher. norweb.se/introEN.html.

DETECTION OF MICROCYSTINS AND NODULARIN BY ENZYME-LINKED IMMUNOSORBENT ASSAY, PROTEIN PHOSPHATASE INHIBITION ASSAY AND HPLC: COMPARISON OF METHODS

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Methods such as enzyme-linked immunosorbent assay (ELISA) and protein phosphatase inhibition assay (PP1) have recently been developed for the detection of cyanobacterial hepatotoxins, i.e. microcystins (MCYST) and nodularin. We evaluated the suitability and the repeatability of these methods to detect MCYST-LR, MCYST-RR, their demethyl variants, and nodularin with pure toxin standards, laboratory cultures of cyanobacteria and natural water samples. The samples were also analysed by two different laboratories with HPLC, and the results obtained were compared. ELISA and PP1 both proved suitable for detecting MCYSTs and nodularin. For most of the MCYST-types the results correlated well, and the concentrations were similar. The results also correlated well with the HPLC analysis after the sample treatment was optimised. No significant difference was observed between the HPLC results of the two different laboratories when the same quantitative standard MCYST-LR was used. PP1-assay gave lower concentrations than ELISA and HPLC for some MCYST-types, which suggests that not all MCYSTs bind to the PP1-enzyme to the same extent. Since several different MCYST-types exist and the methods to detect them differ in principle, it is advisable that a variety of methods should be used when analysing MCYST concentrations from an unknown sample. The study also emphasises the importance of reliable quantitative toxin standards.

HOW AN ALGICIDAL BACTERIUM (*ALTEROMONAS* SP.) KILLS *HETEROSIGMA AKASHIWO* (RAPHIDOPHYCEAE) AND OTHER ALGAE ?

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Among many *Heterosigma akashiwo*-killing bacteria isolated from Hiroshima Bay in 1994 and 1995, an *Alteromonas* sp. strain GY9501 is considered to dominantly influence rapid termination of *H. akashiwo* bloom .GY9501 has a high algicidal activity upon dinoflagellate *Gymnodinium mikimotoi* and *Heterocapsa circularisquama*, beside *H. akashiwo*. A result of an experiment using two component cultivation system suggested that GY9501 kills the algae through unknown algicidal substances. Strong algicidal activity against *H. akashiwo* was observed in GY9501-*H. akashiwo* co-culture filtrate. After ultrafiltration of the filtrate, *H. akashiwo*-killing substance (HAK) existed in 3,000-10,000 MW fraction (HAK-1) and less than 1,000 MW fraction (HAK-2). HAK-1 is heat-stable and has weak algicidal activity on *G. mikimotoi, H. circularisquama*, and other four red tide causing algae. HAK-1 didn't exist in GY9501 monoclone culture in a pepton medium nor in a *H. akashiwo* culture filtrate including extracellular organic matter.

DOMOIC ACID AND METALS: THE ROLE OF DOMOIC ACID IN COPPER HOMEOSTASIS IN *PSEUDO-NITZSCHIA* SPP. FROM MONTEREY BAY, CA

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Specific environmental cues responsible for stimulating the accumulation of domoic acid (DA) in *Pseudo-nitzschia* spp. are poorly defined, although some evidence indicates that production of DA can be stimulated by exposure of cells to elevated metal (Li) concentrations (Subba Rao et al. 1998, P.S.Z.N. Mar. Ecol. 19:31). Other algal species have been observed to produce the amino proline (an structural analogue of DA) in response to elevated copper (Cu) concentrations suggesting a potential complexation strategy, since proline exhibits chemical characteristics found in many unidentate ligands. As part of our ongoing studies investigating the interrelationship of proline and DA metabolism, we evaluated the response in DA pools during exposure of several *Pseudo-nitzschia* species to varying total Cu availability, ranging from limitation to toxicity. Copper enrichment experiments revealed that *P. multiseries* strain MU5 increased cell yield by ca. 2-fold when cultured in elevated Cu conditions (0.16 to 16 μM Cu+2) compared to standard f/2 (0.03 μM Cu+2); *P. australis* strain AU43 did not exhibit this trend. In both species, the production of DA was not stimulated by exposure to elevated Cu concentrations. However, the difference in the growth repsonse between the two species was associated with MU5 maintaining a 100-fold higher DA content, relative to the total amino acid pool, compared to AU43. As domoic acid is comprised of three carboxylic acid groups, a prolyl ring conjugated to an isoprenoid side chain, it is predicted that DA will exhibit enhanced chelation capacity for copper (and other metals) relative to proline, however, stability constants with respect to copper have not yet been measured for this compound. Flow injection analysis with chemiluminescent detection will be used to measure the affinity constants for DA-Cu interactions. The potential role of DA in intracelluar copper homeostasis in *Pseudo-nitzschia* spp. will be discussed.

SPATIAL AND TEMPORAL DISTRIBUTION OF *GYMNODINIUM CATENATUM* AND SHELLFISH TOXICITY FROM 1989 – 1998 IN THE NORTHEASTERN COAST OF SUCRE STATE, VENEZUELA.

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Gymnodinium catenatum was detected in the Venezuelan coasts during the 80's, with a first bloom observed in 1989. Since then a monitoring along the northeastern coast of Sucre State and the Gulf of Cariaco was caried out to detect its presence and to measure environmental parameters (temperature and salinity) that may be associated with its appearance. *Gymnodinium catenatum* has been observed every year along the coast, with maximum abundance between June and October. Blooms of *G. catenatum* (> 1.000 org. ml-1) have only been observed in the northeastern coast during the rainy season (July – Nov.) when water temperature was above 25 C and salinity was 36,5 to 38 Å. The smallest abundance (< 100 org. ml -1) occurred from Dec. – June in a wide range of temperature (22 – 28 C) and salinity (33,2 – 39 Å). It was less abundant in the Gulf of Cariaco and has not formed blooms yet. The dynamics of the marine environment at the north coast seems to favor the growth of *G. catenatum* populations, perhaps due to a greater availability of nutrients in the water. Shellfish toxicity not always coincided with the presence of *G. catenatum* and showed different PSP levels. The relationship between abundances of *G. catenatum* and toxicity in shellfish will also be discussed.

HARMFUL MICROALGAE AS TUMOR PROMOTERS?: CURRENT STATUS IN MARINE ENVIRONMENTS

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Harmful microalgae can produce potent toxins that cause acute mortality in humans and aquatic animals. Because the occurrence of toxic or harmful algal blooms (HABs) tends to be acute and results quickly in shellfish poisoning events or mass mortalities of aquatic organisms, emphasis has been on studying their short-term effects. The chronic harm from biotoxins produced by planktonic or benthic microalgae to aquatic animals or humans is relatively unknown. Some microalgal biotoxins have been experimentally demonstrated to act as tumor promoters in mammals. The prevalence of tumors in aquatic animals has steadily been increasing on a worldwide basis for the last 30 years, yet biotoxins are rarely considered as potential tumorigenic agents. Because there is no information on the fate and effects of natural tumor-promoting compounds in aquatic systems there has been little focus in this area.

Fibropapillomatosis (FP) in green turtles Chelonia mydas is a debilitating, neoplastic disease that has reached worldwide epizootic levels. FP has been reported principally from tropical areas in the Atlantic Ocean, the Indo-Pacific Region, the Pacific Ocean, and the Caribbean Sea. The etiology of FP is unknown but has been linked to oncogenic viruses. Toxic benthic dinoflagellates (*Prorocentrum* spp.) are not typically considered tumorigenic agents, yet they have a worldwide distribution, principally tropical, and produce a tumor promoter, okadaic acid (OA). *Prorocentrum* spp. are epiphytic on macroalgae and seagrasses that are normal components of green turtle diets. In the Hawaiian Islands, we recently demonstrated that green turtles consume substrates with *Prorocentrum*, and that high-risk FP areas are associated with areas where *P. lima* and *P. concavum* are both highly prevalent and abundant. The presence of presumptive OA in the tissues of Hawaiian green turtles further suggests exposure and a potential role for OA in the etiology of FP.

PFIESTERIA AND *PFIESTERIA*-LIKE SPECIES IN FLORIDA

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A survey to determine the presence and distribution of *Pfiesteria piscicida* and *Pfiesteria*-like species (PLS) in Florida estuarine waters (121 stations) was completed in spring 1999 and is being expanded to include intensive sampling in fall 1999 at "hot spot" areas. Environmental variables including inorganic and organic nutrients, heavy metals, chlorophyll, salinity, temperature, dissolved oxygen, and percent organics in sediments were measured to determine correlations between individual variables and the presence of PLS, most of which are new species but closely related both morphologically and genetically. One group of PLS, cryptoperidiniopsoids, was found throughout the state, but the distribution (based on scanning electron microscopy and molecular probes) is discrete and not continuous. Strains of this group are thought to be nontoxic but need to be tested for the production of bioactive compounds. They occur in mid-Atlantic states as well. Two other new species, one a toxin producer and the other a possible toxin producer, have a very limited distribution so far. *Pfisteria piscicida* has the most limited distribution and was found in only one sample. It is not known whether this strain is toxic, but the site will be intensively sampled to verify its presence and determine sample variability. In addition to the subsequent "hot spot" sampling for *Pfiesteria* and PLS (more than 10 new species), a program involving automated, floating platforms configured with continually recording sensors for salinity, temperature, dissolved oxygen, chlorophyll, turbidity, PAR, current direction and velocity, nitrate, phosphate, and pH and a water sampler has been developed. Platforms such as this will help assess fluctuations in environmental variables and their effects on dinoflagellates and other estuarine organisms. Such automated stations will help temporally define hypoxic events.

IDENTIFICATION OF DOMOIC-ACID PRODUCING *PSEUDO-NITZSCHIA* SPECIES IN AUSTRALIAN WATERS

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Extending from an earlier taxonomic survey of *Pseudo-nitzschia* species occurring in Australian water by Hallegraeff (1994), phytoplankton samples were collected from Tasmanian, South Australian, Victorian, New South Wales, Western Australian and Queensland marine and estuarine waters. Single cells of *Pseudo-nitzschia* spp. were isolated from samples and grown in culture. Using a combination of light microscopy, TEM, and rRNA fluorescent probes, cultures were identified and then tested for domoic acid production. Alternatively, preserved field samples were cleaned and observed using TEM.

This study has identified for the first time the presence of *Pseudo-nitzschia australis* (a known domoic acid producer) in Tasmanian, Victorian and New South Wales waters. The occurrence of *P. multiseries* at six sites along the New South Wales coast was noted. Other species identified include *P. pseudodelicatissima, P. pungens, P. delicatissima, P. fraudulenta, P. subfraudulenta, P.subpacifica, P. heimii* and *P. cuspidata.* The present survey confirmed earlier results that the dominant bloom-forming species in Australian waters are *P. pseudodelicatissima* and *P. pungens.* Preliminary analysis using ELISA has confirmed significant levels of domoic acid in extracts from all four *P. australis* cultures tested. Of the five *P. pseudodelicatissima* cultures tested, one produced detectable levels of domoic acid. No toxin was detected in any of the cultures of *P. pungens, P. delicatissima* and *P. fraudulenta* tested. Efforts to isolate cultures of *P. multiseries* have thus far been unsuccessful.

The regular presence in Southern Australian waters of low concentrations of *P. australis* and P. multiseries highlight the need for *Pseudo-nitzschia* and domoic acid monitoring programs to be set up in marine farm areas.

A NEW, ADVANCED METHOD FOR *IN VIVO* STUDIES ON THE INDIVIDUAL BEHAVIOUR AT UPTAKE AND ELIMINATION OF PARTICLES IN THE BLUE MUSSELS, *MYTILUS EDULIS,* USING GAMMA CAMERA TECHNIQUE.

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Suspension-feeding bivalves, such as mussels and oysters, are abundant species in many coastal and estuarine waters and are also cultured and harvested for human consumption. The bivalves have a large filtration capacity and are able to accumulate high numbers of microorganisms and are therefore frequently involved in food-borne diseases. To develop monitoring programmes, a lot of information is needed to understand the uptake, retention time and depuration routes for different compounds. The individual variances of the contents of both alga-toxins and pathogenic microbes in bivalves are well known but good explanations for these observations remain. The aim of this study was to develop a laboratory model that can be used to quantify the individual variation in uptake and transit time for particles in the bivalves. The principle of the method is to use particles labelled with a gamma-radionuclide which is taken up by the organism through its normal feeding route and during the experiment visualise the amount of particles and their distribution within the mussel. By outlining the region of interest of the image displayed, the values of radio-activity from the certain area will be measured. In this study, the mussels were given so called microspheres, (non-degradable latex particles, 15 μm in size, labelled with 57Co). The region outlined to be measured was the stomach-area. The accumulation of radioactivity in this region was calculated as uptake and the reduction was calculated as the elimination. The uptake of the microspheres was shown to be comparable among the 21 mussels included in the study, while the variance of the elimination between individuals was high. In order to investigate the variances within individuals, a number of four mussels were randomly chosen for repetitive measurements of uptake and elimination. The temporary variance of the transit-time within a mussel was shown to be as high as the variance between individuals. Consequently, the individual variances, in terms of alga-toxins or harmful microbes in mussels, could be explained by the arrhythmic activity in the digestive glands. The experiment was carried out independent of diurnal and environmental conditions like temperature, salinity and food availability, indicating that neither of these factors was predominating in regulating the activity.

The study showed that non-degradable labelled particles could be used as markers for uptake and transit times as stated above. The method is non-destructive and thus allows you to do repeated measurements on the same individual. The gamma camera technique could also be used to study biodistribution of radio-labelled organisms such as phytoplankton and bacteria. Particles of various sizes and shapes could be used and two different particles labelled with non-interactive radio-nuclides could be measured in parallel.

MANAGING POTENTIALLY TOXIC CYANOBACTERIA IN A BALTIC COASTAL AREA BY ADJUSTING NITROGEN AND PHOSPHORUS LOAD

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The Baltic Sea is one of the largest brackish water areas on Earth and also one of the few brackish water areas where diazotrophic cyanobacteria are an important component of the phytoplankton. Diazotrophs are common in the southern part, the Baltic Sea proper, where winter surface layer inorganic N/P ratios are well below the Redfield ratio, suggesting predominant N-limitation of phytoplankton growth. Nitrogen fixation is here considered one of the largest sources of new nitrogen, after land run-off but similar to atmospheric deposition.

In Baltic Sea proper coastal areas, nutrient discharges from sewage treatment plants with efficient P-removal have high N/P loading ratios that bring about P-limitation of phytoplankton growth in the discharge area. As a result, diazotrophic cyanobacteria are virtually absent in the phytoplankton.

Here we report results from a study in Himmerfjärden Bay, a northern Baltic proper coastal area, where nitrogen discharges from the Himmerfjärden STP were recently reduced by more than 80% by the introduction of a fluidised bed technique. From 20 years of previous results, we predicted that conditions favouring diazotrophs would result if nitrogen discharges were reduced to one third or less. In practice an increased biomass of the heterocystous filamentous cyanobacterium *Aphanizomenon* sp. resulted. The toxic species *Nodularia spumigena* known to form extensive surface accumulations in the coastal area outside and in the offshore Baltic Sea proper, has so far been virtually absent.

Our results are being used to tailor the management of nutrient discharges from the Himmerfjärden STP so as to avoid toxic cyanobacterial blooms, and will gradually be used also to adjust nitrogen reduction to avoid inducing nitrogen fixation in summer.

ANALYSIS OF THE FRESH WATER CYANOBACTERIAL TOXINS FROM A FISH FARM IN NSW,AUSTRALIA.

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Since Spring 1995, blooms of cyanobacteria have been observed at a fish farm in NSW, Australia and during summer cyanobacterial blooms often dominate the flora of ponds. The relationship between elevated levels of phosphorus in the feeds and the cyanobacterial blooms has been observed. The various types of cyanobacteria that were observed are: *Microcytis aeruginosa, Microcytis flos-aquae, Oscillatoria* spp. and *Arthrospira massartii*. Solid Phase Extraction cartridges, molecular cut-off membranes and HPLC technique were used to analyse and detect the presence of cyanobacterial toxins. Initially, Microcystin-LR and either Microcystin-RR or Nodularin were detected. The toxicity of toxins were also tested. There were no mortalities of silver perch reported. Perhaps the fish were either unaffected by blooms, or had the ability to break down the toxins. Mouse bio-assay tests showed that extracts were toxic. Autopsies of mice revealed that the toxins caused liver and kidney malfunction: livers were enlarged and heavily congested, kidneys were also congested. Prolonged exposure of farm workers to aerosols or contaminated water could be a health risk.

PARALYTIC SHELLFISH TOXINS OF THE MUSSEL, *MYTILUS EDULIS* AND THE TOXIC DINOFLAGELLATE, *ALEXANDRIUM TAMARENSE* FROM CHINHAE BAY OF KOREA

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Toxicity and toxin profiles of the mussel, *Mytilus edulis* and the toxic dinoflagellate, *Alexandrium tamarense* in Masan (Stns M) and Chilchundo (Stns C) of Chinhae Bay, Korea were investigated weekly from March to May in 1996 (Stn M1 and C1) and 1997 (Stn M2 and C2). *Alexandrium tamarense* was more abundant at Stn M1 in 1996 and at Stn C2 in 1997, while density of total phytoplankton was always higher at Masan. Population of *A. tamarense* always occupied larger portion among total phytoplankton at Chilchundo than at Masan. In 1996, the peak of toxicity in the mussels was recorded when *A. tamarense* was abundant in both stations. Maximum toxicity of *M. edulis* was largely different between the stations, much higher at Stn C1 (560 MU g-1) than that at Stn M1 (22 MU g-1). Toxicity in cultured strains of *A. tamarense* at Stn C1 was higher in general than that at Stn M1, ranging from 1.0 to 109.8 MU 10-6 cells-1 and from 3.8 to 59.8 MU 10-6 cells-1 at Stn C1 and Stn M1, respectively. Toxin profiles of the strains showed that C1-C2 and GTX1+4 were the major components, while GTX2+3, GTX5, neoSTX and STX were the minor. In 1997, the mussel toxicity was also much higher at Stn C2 than that at Stn M2. Maximum toxicity in *M. edulis* and peak abundance of *A. tamarense* at two stations were correlated, showing the time lag of 1-2 weeks. Toxicity of the size-fractionated phytoplankton (10-100μm) ranged from 0.002 to 0.073 (MU^{-1}) and from 0.004 to 0.029 (MU^{-1}) at Stn C2 and Stn M2, respectively. PSP causative organism found in size-fractionated phytoplankton was only *A. tamarense*. Size-fractionated phytoplankton showed toxin profiles of C1-C2 only at both stations. However, PSP toxins in *M. edulis* consisted of 10 components including C1-C2, GTX1-5, dcSTX, STX and neoSTX. The results showed that toxicity of *M. edulis* was conspicuously originated from *A. tamarense* blooming in Chinhae Bay, while biotransformation may cause the relative increase in carbamate toxins in the mussels. Although feeding kinetics of the mussels was beyond the subject of this study, higher portion *of A. tamarense* population and lower density of total phytoplankton both may explain that higher ingestion rate of the mussels on *A. tamarense* at Chilchundo led to the higher toxicity.

COMMUNITY STRUCTURE OF FLAGELLATES AND DYNAMICS OF RESTING CYSTS IN KAMAK BAY, KOREA

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To investigate the seed population of red-tide causative organisms, community structure of flagellates and dynamics of resting cysts were monitored monthly from May 1997 to June 1998 at five stations in Kamak Bay, where red-tide occurs year by year in summer in Korean waters. A total of 58 flagellate species were identified including 56 spp. Of dinoflagellate and 2 spp. of raphidophycean. Genus *Protoperidinium* occupied 25.0% of total species number and the highest at station 1 located in most inner bay. Standing crops of dinoflagellate were a maximum of 193,642 cells L-1 in June 1998. Dominant species comprised *Prorocentrum dentatum* in June 1997, and *Prorocentrum minimum* and *Ceratium furca* from May to June 1998. Raphidophycean appeared in a few stations when water temperature increased from May to June 1998. Resting cysts comprised 31 dinoflagellate cysts, representing 13 genera, 27 species, 4 unidentified, and 1 *Chattonella* cyst of raphidophycean. The abundance of resting cysts varied extensively by month: total cysts ranging from 457 to 1,048 cysts cm-3, living cysts from 144 to 564 cysts cm-3 and empty cysts from 313 to 662 cysts cm-3. Living cysts constituted 25.0-55.9% of total abundance and their ratio increased in winter. The abundance of the living cysts showed the significant relationships to water temperature (r2=0.44) as well as to the seasonal succession of planktonic cells in the water column. Only a few livinig *Chattonella* cysts were found from July to August and from January to February, whereas empty cysts appeared in March and April. Of dinoflagellate cysts 17 heterotrophic species were included, comprising 54.8% of total species number and showing higher abundance of 50.4-54.6% in inshore than that of 29.0-36.0% in offshore. This means composition ratio of heterotrophic cysts might be closely related to polluting degree in such frequently red-tide occuring area.

THE GLOBAL BIOGEOGRAPHY OF THE GENUS *ALEXANDRIUM*

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In the past few decades, the incidence of Paralytic Shellfish Poisoning (PSP) and the geographic range of the toxin-producing dinoflagellate genus *Alexandrium* have both increased worldwide. Genetic analysis of populations of *Alexandrium* can help to explain their origin, and thus the mechanisms for dispersal (both natural and human-assisted). Prior work has shown that analysis of LSU rDNA is an effective tool for revealing genetic diversity and phylogenetic relationships in Alexandrium that are not evident from morphology.

This study used an LSU rDNA-based RFLP assay with a broad set of isolates, representing the coastal areas of South America, Africa, New Zealand, Europe and the Mediterranean, in addition to new isolates from previously studied areas. Several novel ribotypes were found within both the *tamarensis* and the *minutum/lusitancum* groups. Isolates of *A. margalefii, A. pseudogonyaulax* and *A. ostenfeldii* all displayed unique ribotypes. In accordance with prior work, ribotypes do not reflect morphospecies; geographic origin is a better indicator of phylogeny than morphology. For all species, each ribotype contains either toxic or non-toxic isolates but not both. For isolates displaying new ribotypes with this RFLP assay, a portion of the LSU rDNA was sequenced. These were placed into phylogenetic context with previously sequenced ribotypes.

These results offer additional insights into the global distribution and dispersal of *Alexandrium*. Within the broadly distributed ribotypes, patterns are revealed that are suggestive of both natural and human-assisted dispersal. Other ribotypes thus far have been seen in single regions, likely representing isolated discrete populations.

BACTERIAL:DINOFLAGELLATE INTERACTIONS; INVESTIGATIVE MICROSCOPY OF *ALEXANDRIUM*

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The association of bacteria with dinoflagellates has been a neglected field of study which has gained prominence in recent years because of the possible role of bacteria in toxin synthesis. A number of dinoflagellates undergo sexual reproduction, passing through various life-cycle stages in addition to the vegetative form. The presence of bacteria within dinoflagellates has been well established but their presence throughout the dinoflagellate life-cycle has not been investigated. Using cultures of *Alexandrium (A. tamarense, A fundyense*) the association of bacteria and various vegetative growth phases (lag, log, stationary) and sexual life-cycle stages (planozygote , hypnozygote) was investigated using SEM, TEM and epifluorescence microscopy. Bacteria were found to be associated with the surfaces of vegetative cells, planozygotes and hypnozygotes. TEM showed bacteria to be present within all vegetative growth phases, as well as in the sexual planozygote and cyst. The presence of intracellular bacteria in the vegetative growth phases was confirmed using DAPI and SYBR green staining combined with epifluorescence microscopy. The ubiquitous presence of bacteria in *Alexandrium* life-cycle stages suggests they may play a role in its biology and ecology.

ECOLOGICAL CHARACTERIZATION OF A WIDESPREAD RED TIDE IN SOUTH CAROLINA ESTUARIES: A NEWLY OBSERVED PHENOMENON.

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Prior to 1998, the only published record of a harmful algal bloom (HAB) in South Carolina estuarine or marine coastal waters was a 1988 *Gymnodinium breve* red tide that originated in the Gulf of Mexico and was transported with the Gulf Stream to continental shelf waters off North Carolina and then southward to South Carolina nearshore waters. In the spring of 1998, a different dinoflagellate (tentatively identified as *Peridinium* sp.) formed a red tide in Bulls Bay near McClellanville, South Carolina, the first documentation of a red tide localized to South Carolina estuarine waters. In the spring through summer of 1999, the dinoflagellate formed red tides at several sites in South Carolina estuaries (North Inlet, Bulls Bay, Broad Creek/Hilton Head Island), commonly comprising > 95% of the total phytoplankton biomass, and at times reaching > 100,000 cell ml-1. Results from field monitoring of physicochemical and microbial food web properties conducted prior to and during the bloom suggest a potential relationship of bloom formation with elevated dissolved organic phosphate concentrations. These and other aspects of "*Peridinium* sp.'s" ecophysiology will be presented, based, in part, on photosynthesis vs. irradiance relationships and ^{15}N uptake kinetics (urea, NH4, NO3) of ambient populations. The recent and recurrent (1998 and 1999) appearance of this widespread (in South Carolina estuaries over 100 miles apart), long-lasting (from spring through early summer), and often intense (turning the water a deep orange color), red tide raises important issues regarding the condition of South Carolina estuaries (e.g. is this phenomenon a signal of changing water quality?). Hypotheses for ecophysiological bases of "*Peridinium* sp." bloom formation will be presented.

INGESTION AND ABSORPTION EFFICIENCY OF SCALLOP (*CHLAMYS NOBILIS*) AND CLAM (*RUDITAPES PHILIPPINARUM*) ON A TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE*

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Paralytic shellfish poisoning (PSP) toxins can be accumulated by bivalves through feeding process, therefore knowledge on the feeding and assimilation of PSP toxin containing algae are critical for understanding the kinetics of PSP toxins in these bivalves. In South China Sea, it has been documented that scallop *Chlamys nobilis* has much higher PSP toxin burden than the clam *Ruditapes philippinarum*. Experiments were therefore carried out to assess whether the difference of toxin burden between these two bivalves was due to the difference in feeding and assimilation. In the mixed diet of *Alexandrium tamarense* (a PSP toxin producing dinoflagellate) and *Thalassiosira pseudonana* (a non-toxic diatom), the two bivalves exhibited the same maximum filtration rate (equivalent to about 50% of the tissue dry weight per day) at 500 cells/ml of *A. tamarense*. At 100 cells/ml of A. tamarense, the maximum clearance rate of scallop (11 L/g/h) was significantly higher than the clam (7 L/g/h). Furthermore, the clams were able to produce pseudofeces at a lower cell density than the scallops. However, we found that the clams were unable to selectively exclude the toxic dinoflagellate by pseudofeces production. In scallops, both the assimilation efficiency (AE) and the gut passage time (GPT) were similar between *A. tamarense* and *T. pseudonana*. Clams however had a higher AE on *T. pseudonana* than on *A. tamarense*, but the GPTs were comparable for both algal diets. In general, AE decreased with increasing concentration of *A. tamarense*. Thus, it is likely that a higher PSP toxin level in scallops was due to: (a) a higher clearance rate; (b) no pseudofeces production at a relatively high algal density; and (c) slower detoxification, which is currently under investigation.

PHYSIOLOGICAL AND MOLECULAR CHARACTERISTICS OF CELL CYCLE AND GROWTH OF *PFIESTERIA* AND RELATED DINOFLAGELLATES

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Classical predator-prey oscillation was observed for *Pfiesteria piscicida* and related species with algal food species, strongly suggesting roles of algal food availability in regulating dynamics of these dinoflagellate populations. Several cell division cycle (CDC)-related genes showed close relatedness to animal and higher plant counterparts. During oscillation of growth rate, expression of these CDC genes displayed growth rate-dependent fluctuation. The potential of these genes as markers for studying in situ growth rate and population dynamics of these species will be discussed.

VARIATION IN CELLULAR CONTENTS OF PSP TOXINS IN A BLOOM OF *ALEXANDRIUM MINUTUM* IN BAYONA BAY (NW SPAIN).

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Alexandrium minutum, a PSP producing species which recurrently blooms in the Bayona Bay, produced a toxic episode in September 1998, that was detected through mouse bioassays of mussels from the area. Bayona bay is a small inlet (ca. 4x3 Km) subsidiary of the Ría de Vigo. As soon as the toxicity in mussels was detected, a water sampling, that included six different sites in the bay, was started and maintained with a periodicity of two-three days until the nearly disappearance of the *Alexandrium* population, with the objective of determining the possible differences in toxin contents per cell and also those in toxin profiles, in and during the bloom. The obtained water samples were filtered through Whatman GF/C filters that were afterwards extracted. The PSP toxins were analysed by HPLC. The toxin profiles found were similar to those of the culture AL1V of the Instituto Español de Oceanografía, because they only contained GTX1 to 4 in significant amounts, although their relative proportions were slightly differents. Large differences were found both spatially and temporally. The total toxin concentration per cell varied more than one order of magnitude. The variation in toxin contents per cell, had a spatial component, changed by a factor of 5.2 and a temporal component, changed by a factor of 7.8. The possibility that those changes were produced by the environmental conditions was also studied.

WATER COLUMN NUTRIENT CHARACTERISTICS ASSOCIATED WITH BLOOMS OF THE NUISANCE ALGA *AUREOCOCCUS ANOPHAGEFFERENS*

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Shallow coastal bays along the eastern United States have been intermittently plagued by blooms of the nuisance alga *Aureococcus anophagefferens*, which impacts the ecosystem by leading to substantial loss of shellfish and seagrasses. This study focused on comparing the water column nutrient characteristics of 1999 Aureococcus blooms in Long Island, New York and Maryland coastal bays. During July and August, weekly samples were collected and analyzed for ambient nutrients, both inorganic and organic, biomass parameters, and nitrogen enzyme activity. Within the context of this study, the threshold for an *Aureococcus* bloom was set at 100,000 cells/ml. Aureococcus densities were not significantly correlated to inorganic nutrients (N or P) in either system; however, sites with high densities of *Aureococcus* had a unique signature in terms of organic C, N, and P ratios that was different from sites with low *Aureococcus* densities. High densities of Aureococcus were associated with greater than Redfield DOC:DON ratios and DON:DOP ratios that approached the Redfield ratio. Pairwise comparisons of the same set of stations with high and low *Aureococcus* densities found these differences to be significant ($P < 0.05$). Absolute concentration data suggest that the variation in these ratios is associated with changes in all three organic nutrient pools, but in particular DOC and DOP. To test this trend further, historical data from Long Island Bays was divided based on this same criteria, and also showed that *Aureococcus* blooms are associated with high DOC:DON and near Redfield DON:DOP ratios. This association of high *Aureococcus* densities and elevated organic nutrients has support from several lines of physiological evidence. These data lend support to the growing recognition of the role of organic nutrients in either or both the initiation and maintenance of *Aureococcus* blooms and underscore the need to better understand the dynamics of dissolved organic matter cycling.

GEOGRAPHIC DISTRIBUTION AND TAXONOMY OF POTENTIAL TOXIC CYANOBACTERIAL STRAINS IN MOROCCO

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In Morocco reservoirs and shallow lakes poisoning events of fish, aquatic birds and livestock have been observed during Summer. In all cases, the mortality reasons have not been confirmed, the toxic cyanobacteria strains that were abundant in these water bodies has been suspected. In order to establish a screening of potential toxic cyanobacterial strains in Morocco, the authors started taxonomic and ecological studies since 1994 by collecting samples from various lake reservoirs and ponds. The results show that 14 out of 26 lakes reservoirs used for human water supply contained at least one species of planktonic cyanobacteria, where the genus *Microcystis* was dominant (*M . aeruginosa f. aeruginosa; M. aeruginosa f. flos-aquae; M. ichtyoblabe; M. pulverea f. delicatissima*) and associated with *Planktothrix, Anabeana*, or *Phormidium* species. Among of 150 cyanobacterium taxa identified from Moroccan freshwater ecosystems, 34 species are potentially toxic. In particular conditions, as in brackish Oued Mellah reservoir, the blooms of *Microcystis ichtyoblabe* was substituted by those of *Aphanizomenon flos-aquae* associated with *Planktothr*ix. By using electronic microscopy, a comparison of cytology between the most frequent cyanobacetrium (*M. aeruginosa f. aeruginosa* and *M. ichthyoblabe*) cells has been performed. In term of this study, a map of geographic distribution of all potentially toxic cyanobacteria species identified from Moroccan water bodies will be presented and discussed.

FLUORIMETRIC TECHNIQUE FOR QUANTITATION OF PARALYTIC SHELLFISH POISONING (PSP) TOXINS BY USING EXCITABLE CELLS

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Accumulation of toxins causing paralytic shellfish poison (PSP) in mollusks is a seasonal phenomenon that occurs with great variability. Since the ingestion of these toxins may cause death, PSP toxin levels in seafood products are estimated using the standard AOAC mouse bioassay to prevent the consumption of contaminated bivalves. However because of limitations of this bioassay (high variability, low sensitivity, limited sample throughput and use of live animals) other methods of monitoring PSP toxin levels are clearly needed.

We proposed a fluorimetric technique based on registration of changes in membrane potential of excitable cells (human neuroblastoma) for quantitation of PSP toxins. The method of detection of membrane potential involves the steps of a) incubating neuroblastoma cells with the fluorescent dye bis-oxonol, whose distribution across the membrane is potential-dependent, and measuring intracellular dye concentration b) depolarization of the cells with veratridine a sodium channel-activating toxin which enhances sodium ion flux c) inhibition dose-dependent of depolarization with PSP toxins (sodium-blocking toxins). Finally we relate percentage of inhibition with toxin concentration. This is a rapid, reliable and specific method of monitoring PSP toxin levels in samples from seafood products.

TOXIC DINOFLAGELLATES AND TOXIN STUDIES IN TAIWAN

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Thirty-five clones, belonging to twelve species of dinoflagellates were isolated from planktons in brackish water ponds or from the wash-off epiphytes of Sargassum spp. These isolates were identified as species of *Amphidinium carterae, A. klebsii, Gyrodinium instriatum, Coolia monotis, Gambierdiscus toxicus, Ostreopsis lenticularis, Prorocentrum lima, P. mexicanum, P. mininum* and some unknown species belonging to Gonyaulax, *Gymnodinium* and *Prorocentrum*. Methanol extracts of the filtered cells were diluted with water to make 60% methanol solution and partitioned with n-hexane and chloroform. Both chloroform and aqueous fractions were screened for their toxicity in mouse (i.p. injection) and brine shrimp (solution). Chloroform extracts of *G. toxicus, O. lenticularis, P. lima, A. klebsii* and some clones of *A. carterae* and *Prorocentrum* sp. showed mouse toxicity. Brine shrimp larvae also responded to the methanol extract of these isolates in variable extents. Among them clone #2 and #4 of A. carterae, and clone #1 of *A. klebsii* were found the most toxic ones. It was also found that the chloroform extract of *P. lima* and *G. instriatum* exhibited toxicity to brine shrimp. Toxins belonging to different categories in these algal isolates were studied for their chemical nature. From cell cultures of one toxic *P. lima* clone eight okadaic acid (OA) analogues and derivatives were isolated. Among them, okadaic acid (OA), methyl ester of OA, dinophysistoxin-1 (DTX1), and two diol esters of OA, OA-D8 and OA-D9d, were identified by NMR and EI-MS analysis. One of the compound, OA-D9d was recognized as new in the literature. The remaining three compounds were preliminarily identified as new compounds of DTX4 type toxin and their structures remained to be determined. It was also noted that quite a lot of OA and DTX1could be isolated from the cell-free media of *P. lima* cultures. It seems that the free acid form of toxins are easily excreted outside the cells.

GENETIC AND MORPHOLOGICAL VARIATION WITHIN A SPECIES OF *PSEUDO-NITZSCHIA*

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Species from several different phytoplankton groups have been shown to differ genetically between biogeographically different areas. These variations are important when dealing with toxic species, and may sometimes explain differences in toxicity. The variations are also important when considering the potential use of molecular probes for identification of phytoplankton species within groups of organisms that are difficult to identify.

Isolates of *Pseudo-nitzschia delicatissima* from biogeographically different areas of the world were examined using TEM and fluorescent rRNA-probes. Differences in both morphology and probing results will be discussed. The results indicate that probing with fluorescent rRNA-probes might be an easy way of screening for genetic variability. It may also be a means of understanding diversity of toxicity in toxic species.

EFFECT OF SYMBIOTIC BACTERIA ON GROWTH AND TOXIN PROFILE OF TOXIC DINOFLAGELLATE *ALEXANDRIUM MINUTUM* T1

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The symbiotic bacteria of *Alexandrium minutum* T1 were isolated and identified as follow: extracelullar species including *Pasteurella haemolytica, Sphmon pancimobilis* and *Pseudomonas vesicularis*, and intercelullar species including *Pasteurella pneumotropica, Morganella wisconsensis, Flavobacterium oryzihabitans, Pseudomonas pseudomallei* and *Sphmon pancimobilis.* All of them were cultured and determiner to have no PSP-producing ability identified by HPLC analysis. The cell toxicity of *A. minutum* did not decrease even the culture medium were added with antibiotics. When the dinoflagellate were cultured together with symbiotic bacteria, *M. wisconsensis, S. pancimobilis* or *P. pseudomallei*, the growth of *A. minutum* was better then that with no bacteria at the beginning, but the maximun cell number was lower. The cell toxicity of *A. minutum* cultured with bacteria was similar to that of *A. minutum* cultured without bacteria from lag phase to stationary phase, but it is lower after stationary phase.

NOVEL EXOTOXIC PRINCIPLES PRODUCED BY THE RED TIDE DINOFLAGELLATE *ALEXANDRIUM MINUTUM*

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Endocellular content of Paralytic Shellfish Poisons (PSP) and exocellular toxicity of the growth medium were investigated in the red-tide dinoflagellate *Alexandrium minutum* over the growth cycle in 15L batch cultures. Total sodium channel blocking (SCB) activity (neuroreceptor binding assay) and HPLC profiles of individual gonyautoxin (GTX) 1-4 components (endocellular only), as well as toxicity of the cell-free medium to *Artemia* were examined. Peaks in toxicity of the exocellular medium coincided with peaks in the endocellular toxicity of GTXs, both occurring in early to late lag phase and declining with culture age. SCB toxicity of the medium was found to be approximately 20 times higher (4-10 pg STX equivalents cell-1) than the endocellular SCB toxicity (169-610 fg STX equiv. cell-1). Endocellularly, GTX2 and GTX3 were dominant early in culture growth (36-64 mole%, epimeric total on day 48) but as time progressed GTX1 and GTX4 became increasingly important (58-93 mole %, epimeric total on day 182). Antibiotic treatment of dinoflagellate cultures reduced bacterial levels by up to 93% but this did not appear to affect SCB toxicity, although it did decrease toxicity of the exocellular medium to *Artemia*, which was highest late in culture growth. Toxicity of the *A. minutum* cell-free medium towards *Artemia* did not appear to be correlated with exocellular or endocellular total SCB toxicity nor was it correlated with any individual toxic GTX fraction. It is concluded that, in addition to a sodium channel blocking agent, *A. minutum* is producing another toxin capable of killing *Artemia*, but which is not PSP. Furthermore, this exotoxic principle is heat labile (reduced toxicity to *Artemia* above 80C), but is not a gonyautoxin or brevetoxin.

ELIMINATION AND DIFFERENTIAL TRANSFORMATION OF YESSOTOXIN BY THE GREENSHELL MUSSEL *PERNA CANALICULUS* AND THE BLUE MUSSEL *MYTILUS GALLOPROVINCIALIS*

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Greenshell (*Perna canaliculus*) and Blue(*Mytilus galloprovincialis*) mussels were experimentally contaminated with yessotoxin (YTX) by feeding with a culture of the dinoflagellate *Protoceratium reticulatum*. Tissue localization of YTX and its derivatives was measured by HPLC- FD and their disappearance from the shellfish was monitored over a 10 day depuration period. After feeding, only YTX could be detected in Greenshell mussels whereas in Blue mussels 45-hydroxy yessotoxin (45- OH-YTX) was the predominant molecular species observed, comprising >90% of the total toxin present. Neither derivative could be detected in tissues other than the digestive gland. The slow rate of disappearance of YTX from Greenshell mussel digestive glands over time (0.06 µg YTX g-1 day-1) was comparable with the rates observed in naturally contaminated shellfish (0.04 µg YTX g-1 day-1). The rate of disappearance of 45-OH-YTX from Blue mussels was possibly more rapid though wide variation between replicates made this uncertain. This experiment clearly demonstrates that different bivalve species may interact with dinoflagellate polyether toxins in different ways.

RELATIONSHIPS BETWEEN NITROGEN UTILIZATION AND ENVIRONMENTAL CONDITIONS DURING AN *ALEXANDRIUM MINUTUM* BLOOM IN MORLAIX BAY (NW FRANCE).

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Red tides caused by the toxic dinoflagellate *Alexandrium minutum* Halim were observed in the Penzé River estuary (Morlaix Bay, NW France) in the early summers of 1996 and 1997. During these events, maximum motile cell concentrations reached 18.106 and 44.106 cell.l-1 respectively, corresponding to 56% and 97% of the total phytoplankton population, also constituted with diatoms (particularly *Nitzschia* species) and other dinoflagellates (*Heterocapsa triquetra* and Scrippsiella spp.).

Results concerning the environmental conditions including hydrology, dissolved oxygen and nutrient concentrations obtained in 1997 on four transects of 7 stations along the salinity gradient in the estuary will be presented; the environmental changes prior, during and after the bloom will be detailed. During *Alexandrium minutum* proliferations nitrogen uptake (ammonium and nitrate) was measured using 15N tracer techniques. Nitrate and ammonium uptake reached respectively 292 and 95 nmol.l-1h-1 and nitrate constituted the main source of nitrogen, representing up to 75% of the nitrogen uptake.

ASSOCIATIONS BETWEEN *PFIESTERIA*, FISH HEALTH AND ENVIRONMENTAL CONDITIONS IN CHESAPEAKE BAY

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In 1997, three Chesapeake Bay tributaries were affected by toxic outbreaks of *Pfiesteria piscicida*. These findings led to an intensive environmental monitoring program that evaluates physical, chemical, and biological parameters to both protect public health and to further understanding of conditions leading to such outbreaks. This monitoring program also includes an intensive investigation of the prevalence, geographical distribution and pathology of the Atlantic menhaden ulcerative disease that has been associated with *P. piscicida* toxicity. During 1997-1999, associations were found between fish lesions in menhaden and *P. piscicida* in the three areas that experienced outbreaks in 1997 and at least four other locations. Monitoring results suggest that elevated levels of water column nutrients (particularly organic forms), phytoplankton and temperatures, and a salinity range of 3-12 ppt are associated with the coincidence of *P. piscicida* and lesioned menhaden. The volume and timing of freshwater flows into these tributaries is controlling, at a minimum, salinity distributions, nutrient fields and the longitudinal positioning of phytoplankton maxima which may, in turn, be related to interannual differences in menhaden distributions and *P. piscicida*'s presence in the water column. In the Pocomoke River Estuary, the most intensively studied tributary, fish kills, high prevalence of lesions, and the presence of *P. piscicida* occurred in a region of the estuary where a phytoplankton maximum persisted for at least two months during the summer of 1997 and two localized storm events in the Pocomoke watershed maintained relatively constant salinity levels as overall freshwater flows in the region declined. Several alternative hypotheses will be presented concerning these associations and potential cause and effect relationships.

RELATIONSHIPS BETWEEN INTRACELLULAR BACTERIA AND THE BIVALVE KILLER DINOFLAGELLATE *HETEROCAPSA CIRCULARISQUAMA*

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Laboratory of Marine Environmental Microbiology, Division of Applied biosciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan In the embayments of central and western Japan, *Heterocapsa circularisquama* (H.c.) has caused large-scale red tides, which broughton mass mortalities to both natural and cultured bivalves. The alga has bacteria inside the cells and occasionally in their food vacuoles. It is suspected that these bacteria probably give some effects on the algal ecophysiology. Mutual relationships were investigated between the growth of H.c.. and intracellular bacteria with laboratory culture experiments. Five H.c. strains (Y, UA, UB, A, and I) were isolated from different locations. Each H.c. strain culture was established with elaborate micro-pipette washings, nevertheless, each strain culture contains an intracellular and extracellular bacterial population, designated as Yb, UAb, UBb, Ab, Ib, respectively. The extracellular bacteria were presumably released out from the algal cells. The total numbers of bacterial cells in each H.c. culture increased during algal exponential growth phase. According to the final cell yields, the five bacterial populations are divided into three groups, Yb and UAb, Ab and Ib, and UBb group, respectively. In this order, the bacterial growth depended on live algal cells. Growth experiments were carried out on the five H.c. strains with bacteria and a bacteria-free strain, established from the strain UB, under the conditions of five different light intensities and in five different strengths of medium. There appeared to be no significant advantages for the growth and survival of the algal strains with intracellular bacteria, as compared with the bacteria-free strain. Intracellular bacteria, however, might contribute to H.c. populations in some aspects such as life cycle and toxin production.

THE ROLE OF TRACE-METALS ON TOXIGENIC PSEUDO-NITZSCHIA BLOOMS

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Toxigenic *Pseudo-nitzschia* sp. have been linked to amnesic shellfish poisoning events along the California coast. These diatoms can produce the toxin domoic acid (DA), a secondary metabolite whose algal physiological role is presently unknown. Factors triggering *Pseudo-nitzschia* blooms and enhancing the production of DA in nature are poorly understood. We are investigating the potential role of trace metals, both as limiting and/or toxic nutrients, on *Pseudo-nitzschia* population dynamics and toxicity. To date, lab studies have only demonstrated a link between cellular DA production and macronutrient limitation. But standard culturing techniques may inadvertently contaminate the media with trace metals, and thus are inadequate for assessing the role of trace metals on toxigenic *Pseudo-nitzschia* blooms. The chemically well-defined synthetic seawater medium Aquil was modified to optimally culture two *Pseudo-nitzschia* sp., *australis* and *multiseries*, under trace metal clean conditions. The growth rates of these species in Aquil (1.04 +/-0.16 d-1) were comparable to those achieved previously in amended natural seawater (1.25 +/-0.2 d-1). Concentrations of Fe, Mn, and Cu in Aquil media were then altered to determine optimal cellular trace metal requirements. Our preliminary results suggest that these Pseudo-nitzschia sp. have uniquely high Fe and Mn requirements, and that Fe and Mn interact in a synergistic manner. We also examined whether the production of DA by these diatoms is enhanced under trace metal limiting (Fe and Mn) or toxic (Cu) conditions. These findings will potentially allow us to predict the occurrence of toxic bloom events based on trace metal concentrations and their speciation in coastal waters.

VARIATIONS IN CELL DENSITIES OF TOXIC BENTHIC *PROROCENTRUM* ON SEAGRASS BLADES IN TAKLONG ISLAND, GUIMARAS PROVINCE, PHILIPPINES

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The cell densities of benthic *Prorocentrum* on the tip, middle and lower regions of seagrass blades, *Enhalus acoroides*, were investigated during southwest monsoon months, July- August 1999. The seagrass blade samples were taken from two adjacent coves in Taklong Island, Guimaras province wherein one cove is partially drained and the seagrass is exposed during low tide while the seagrass in the other cove is submerged during low tide. The benthic dinoflagellates invariably composed of *Prorocentrum* constituted 2.2 to 63.2 % of total microbenthic cell populations found on seagrass blades. There were six *Prorocentrum* species observed and a toxic species, *Prorocentrum lima*, was dominant during the study. The *Prorocentrum* cell densities on seagrass blades ranged from nil to 994 cell· cm-2. There were significant differences ($p>0.01$) in the *Prorocentrum* cell densities on the three blade regions. Exposure of seagrass during low tide has no significant effects ($p>0.01$) on the *Prorocentrum* populations. However, it was observed that few days after a typhoon in July, there was a marked decrease in *Prorocentrum* densities on seagrass blades. It is likely that a combination of physical factors particularly strong wave action and the separation of withering or decaying tips of seagrass bring about the entry of *Prorocentrum* into the coastal food web.

DINOPHYSIS ACUMINATA DISTRIBUTION AND SPECIFIC TOXIN CONTENT IN RELATION TO MUSSEL CONTAMINATION.

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The lack of correlation between *Dinophysis* cell density and mussel toxin contents is understandable since very often, an instantaneous measure is compared to an integrated value. The dinoflagellate spatial heterogeneity in patches and a possible variability in specific toxin content make difficult the establishment of a quantitative relationship. The different sources of variance have to be estimated and separated. In situ mussel contaminations were carried out in the Bay of Seine (English Channel) during an oceanographic cruise. Batches of mussels attached to a rope were immersed at 5 stations on both sides of the Seine plume, at three different dephs (about -2, -5, -8m tidal average). The area was sampled during the cruise for a description of *Dinophysis* distribution throughout the bay and in the water column. Five days after immersion, mussels were collected and analyzed for their toxin contents. Maximum cell density was observed in the pycnocline with *D. acuminata* (64000 cell.l-1) representing 90% of the *Dinophysis. D. acuminata* was mainly present in the upper mixed layer. Toxin contents in mussels showed a significant decrease with depth. This result shows that the toxicity level in mussels mirorred the average distribution of *Dinophysis* in the bay. In parallel, an analysis of *Dinophysis* spp-enriched fractions collected during the cruise revealed variable A.O. cell concentrations (3 to 50 pg AO.cell-1; cells did not contain DTX1). During the following year, a red-tide *Dinophysis* bloom (up to 1.5 10^5 cell.l-1) occurred in late summer and the experiment could not be repeated entirely. Nevertheless, a number of mussel samples were collected and *Dinophysis* concentrated fractions were analyzed: AO concentrations were very low in all phytoplankton samples and undetectable in mussels. In conclusion, *Dinophysis* spp distribution in the water and variability of its toxicity must be taken into account in order to improve the precision of toxic phytoplankton monitoring and the management of shellfish surveying.

BLOOMS OF *MESODINIUM RUBRUM* AND RESULTING SALMON MORTALITIES AND STRESS DURING 1998 AND 1999 IN THE BAY OF FUNDY, EASTERN CANADA

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Red tides caused by the organism *Mesodinium rubrum* were observed in Passamaquoddy Bay, southwest Bay of Fundy, during the three weeks of late August through mid-September, 1998 and again during one week in mid August, 1999. Brick-red blooms of the planktonic ciliate have been observed in the past in many coastal regions throughout the world, including Passamaquoddy Bay. *M. rubrum* concentrations observed from water samples collected during each of the bloom periods exceeded 1 million cells.L-1 in areas of water discolouration with dense concentrations observed to a depth of 3 m. The dominant organism, *M. rubrum*, represented up to 95% of the total algal population.

During both years, discrete water samples collected for phytoplankton identification and enumeration revealed concentrations of *M. rubrum* greater than 1 million cells*L-1 at various locations in Passamaquoddy Bay. Sigma-t profiles indicated that the water column was stratified during August and well mixed during September. In addition, during 1999 the bloom was studied over a 24 hr period to determine vertical migration patterns; currents were studied; and a CTD depth profiler was deployed to measure temperature, salinity, density, chlorophyll a, and oxygen.

Although *M. rubrum* does not produce a toxin, it is possible for stress and mortalities among aquatic organisms to occur through secondary effects such as asphyxiation as a result of oxygen depletion or gas bubble disease associated with supersaturation. During the red tide of 1998 red tides drifted through a number of salmonid aquaculture sites resulting in low level mortalities during late stages of the bloom. In 1999, when high concentrations were observed for a period of only one week, salmon experienced and exhibited symptoms of stress during the period when elevated oxygen levels of 180% saturation were measured.

BLUE GREEN ALGAE CONTINGENCY PLANNING IN CENTRAL WEST NEW SOUTH WALES

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Blooms of blue green algae continue to create problems in water bodies throughout New South Wales. To deal with blue green algal blooms, an integrated approach has been adopted by the State Algal Coordinating Committee which incorporates immediate, short, medium and long term solutions (Anon 1992). These solutions address problems arising from algal blooms as well as dealing with the causes of blooms. One aspect of the management of blooms is contingency planning to ensure adequate monitoring, communication and coordination of activities which are needed when a bloom occurs.

This paper outlines the formation of the Central West Regional Algal Coordinating Committee (RACC) and its coverage. The main focus of the paper is to describe recent blue green algal blooms in the Macquarie and Lachlan Rivers and the management responses coordinated by the RACC. In dealing with these two major blue green algae blooms, a number of long term issues were identified which need to be addressed by a coordinated research effort.

DIFFERENTIAL DISPLAY OF GENES EXPRESSED BY SAXITOXIN-PRODUCING DINOFLAGELLATE *ALEXANDRIUM TAMARENSE* CULTURED UNDER VARYING N/P RATIOS

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Saxitoxin-producing strains of *Alexandrium tamarense* have been shown to produce more toxin when the N/P ratio in their culture medium increases. Strain KAC02 isolated from the Baltic Sea and cultured in Norway showed high toxin content at N/P higher than 40:1. This strain was cultured in Vigo under the following N/P ratios in the culture medium: 1.6:1, 6.4:1, 16:1 (Redfield ratio), 40:1 and 160:1. Genes transcriptionally regulated and differencially expressed under the different conditions were visualized with the Differential Display technique, based in the RT-PCR. mRNA was extracted from the cells and reverse-transcribed . The cDNA transcripts were amplified by PCR with 10-mer primers, acrylamide-electrophoresed and silver stained. We avoided working with radioisotopes. This treatment was repeated with the use of different primers and primer pairs for the PCR, thus generating several banding patterns for each mRNA.The differential bands of interest resulting from the different treatments were excised, eluted, reamplified and purified. The purified bands were subsequently cloned and sequenced. There are bands expressed only in either excess or deficiency of N and P, and bands unique to the more nutrient-balanced state. We are in the process of sequencing. Some of the bands are preliminarily thought to code for stress proteins. We also discuss some problems found in the *Alexandrium* mRNA handling.

NUTRIENT RATIOS IN THE NEAR-SHORE WATERS OF THE CATALAN COAST: A MORE REALISTIC SCENARIO FOR THE HABS INCREMENT IN THE NW MEDITERRANEAN SEA.

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Among the causes suggested for the enhancement of Harmful Algal Blooms (HABs) are the important pool of discharged nutrients in the coastal waters and/or the significant changes in nutrient ratios induced by antrophogenic activities. The increase of HABs events that is occurring worldwide has also involved the NW Mediterranean, where there have been recorded numerous dinoflagellate blooms in the last 10 years. The Mediterranean physical-chemical characteristics make bloom occurrences apparently improbable. This traditional statement comes from extrapolating open sea characteristics to near-shore waters where bloom events actually occur and in addition have more repercussions. The NW Mediterranean supports a very high human population (100 habitants/m of coastline in some regions); therefore, near-shore waters are very much subjected to antrophogenic consequences. We present an extensive study of the stoichiometry of dissolved inorganic nutrients along 400 Km of a very much humanised coast (the NE Spanish coast). A general shift in nutrient ratios towards silicate limitation is evident and its implications in dinoflagellate bloom events with the interaction of other factors are discussed.

PARALYTIC SHELLFISH POISONING ON FRENCH MEDITERRANEAN COAST IN THE AUTUMN OF 1998 : *ALEXANDRIUM TAMARENSE* AS A CAUSATIVE AGENT.

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Almost yearly since 1988, summer blooms of toxic *Alexandrium minutum* have occurred along Brittany (N.E. Atlantic). For the first time, during the autumn of 1998, monitoring toxicity tests performed on shellfish collected in the Thau lagoon (W. Mediterranean coast) gave neurotoxicity symptoms on mice. Plankton samples collected, which were almost monospecific, indicated that a dinoflagellate, subsequently identified as *A. tamarense*, was the likely cause. Throughout the toxic event, average cell concentration seldom exceeded 50 000 cells.L-1, despite a peak of 3.5 105 cells.L-1 on November 9th. HPLC analysis showed that plankton and shellfish extracts had a similar toxic profile, which was much more complex (C1,C2, GTX 1/4, 2/3, B1, B2, STX, dc-STX) than that characteristic of *A. minutum* in France (C1, C2, GTX 2/3). Shellfish marketing was banned for almost two months. Shellfish toxicity shows different pattern for the 3 species that were regularly sampled. Using the PSP AOAC mouse-test, a maximum toxicity of 850 μg STX eq./100 g of tissue was encountered in mussels which remain contaminated during all the event, whereas oysters never went above the sanitary threshold (80 μg STX eq./100g), and clams went slightly above. The spatiotemporal distribution of the event throughout the lagoon in these shellfish species monitored is considered in terms of mouse-tests and HPLC results. The latter indicate that *Alexandrium tamarense* is the causative agent.

EFFECTS OF HARMFUL ALGAE ON THE EARLY PLANKTONIC LARVAE OF OYSTER, *CRASSOSTREA GIGAS*

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Effects of harmful algae, *Alexandrium tamarense, A. taylori, Chattonella antiqua, Cochlodinium polykrikoides, Gymnodinium catenatum, G. mikimotoi, Heterocapsa circularisquama, Heterosigma akashiwo*, and *Scrippsiella trochoidea* on the larval pacific oyster, *Crassostrea gigas* were preliminary examined in laboratory in order to clarify the cytotoxicity on early life stages of oysters. Significant harmful effects were found in trochophore larvae exposed to *A. tamarense, A. taylori, G. mikimotoi*, and *H. circularisquama* in cell density of 10^5-10^7 cells/L. Of these, *A. taylori* and *H. circularisquama* kills larval oyster within several hours in cell density of 10^5-10^6 cells/L. In case of exposure to *C. polykrikoides*, although considerable mortality and damage was not found in larval oyster even at 10^7 cells/L exposure, the trochophore larvae exhibited an extreme retardant of metamorphosis to the D-shaped larvae. In contrast, exposure to *C. antiqua, G. catenatum, H. akashiwo*, and *S. trochoidea* did not affect on the trochophore larvae of oyster at all the cell density. As the results we obtained, effect of harmful algae on the larval oyster was extremely varied.

MONITORING AND MANAGEMENT OF CYANOBACTERIA IN QUEENSLAND

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Toxic cyanobacterial blooms in Queensland water storages are common phenomena which pose a potential risk to human health, livestock, and components of the aquatic ecosystem. Generally these blooms occur in the summer months during periods of relatively low river flow, falling storage levels, higher water temperatures, and elevated solar radiation. This also corresponds to the period when these storages are under the greatest demand from both irrigators, and recreational users.

Since October 1997, 47 reservoirs and weir pools across tropical and sub-tropical Queensland have been regularly monitored for the occurrence of cyanobacteria. Results of this monitoring have shown that unlike the seasonal occurrence of cyanobacteria in temperate and sub-tropical regions of Australia, cyanobacterial abundance in much of the tropical regions of the state is aseasonal, hence posing a significant risk year round. To date the potentially toxic species identified in Queensland freshwaters have been *Microcystis aeruginosa* which produces hepatotoxic microcystins, *Anabaena circinalis*, which produces neurotoxins allied with paralytic shellfish poisoning, and *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum*, which produce the alkaloid hepatotoxin cylindrospermopsin.

Management strategies developed include the implementation of routine monitoring programs, development of generic and site-specific contingency plans and the use of internet based information systems for the dissemination of risk-based, hazard assessment information to water users. The success of this approach in providing a rapid and ongoing risk assessment to water users including a mechanism for constant review and adoption of new research findings will be discussed within the Queensland tropical and sub-tropical context.

PHYSICIAN DIAGNOSIS AND REPORTING OF CIGUATERA FISH POISONING IN AN ENDEMIC AREA

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Ciguatera fish poisoning is the most common marine seafood toxin disease worldwide. It is associated with the consumption of large reef fish contaminated with extremely potent natural marine toxins elaborated by micro-algae known as dinoflagellates. Although a reportable disease with a proven acute therapy, Ciguatera is largely under-diagnosed and under-reported in the US. This study presented a classic case of Ciguatera to family medicine physicians in the endemic area of Dade County, FL to evaluate their knowledge of diagnosis, treatment and reporting of Ciguatera.

Of the 78 eligible participants, 36 (46%) participated with 2 refusals and 40 (51%) lost to follow up. The majority of the participants were male (27 (75%)), born in the US (19 (53%) and attending medical school in the US (23 (64%)); the mean years of practice were 20.56+11.66. Although 25(68%) of the participants diagnosed Ciguatera, only 6 (17%) correctly recommended intravenous mannitol therapy as the acute treatment of choice. Almost all the participants (35 (97%)) had heard of ciguatera, but only 23 (64%) had ever diagnosed a case with an average of 0.14+0.42 cases in the past year. Furthermore, only 17 (47%) of the participants knew that Ciguatera was a reportable disease. Foreign-born physicians were significantly more likely to know that Ciguatera was a reportable disease ($p=0.02$); foreign-trained physicians were also more likely to know, although not significantly ($p=0.08$).

This study illustrates that even in an endemic area, Ciguatera is an under-diagnosed, inadequately treated and under-reported disease, especially among US born and US trained physicians.

SUCCESSION BETWEEN *ANABAENA CIRCINALIS* AND *AULACOSEIRA* SPP. IN AUSTRALIAN FRESHWATERS : THE ROLE OF LIGHT AND MIXING

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Anabaena circinalis is a toxic freshwater cyanobacterium responsible for the largest recorded algal blooms in Australian freshwaters. It often dominates the freshwater phytoplankton in Australian waters during the summer months, outcompeting another major bloom forming alga, the diatom *Aulacoseira* spp. From previous field observations it has been hypothesised that this succession is linked to river-flow and is influenced by associated mixing and light availability. The buoyant *A. circinalis* is believed to be able to take advantage of conditions of low flow and stratification, by maintaining a position within the very shallow euphotic zone. Under these same conditions, *Aulacoseira* sinks out of the euphotic zone and loses its ability to compete.

We tested this hypothesis in the laboratory using growth columns measuring 1m x 0.1 m dia. by altering mixing and light regimes with cultures of *A. circinalis* and *Aulacoseira* sp. The use of an attenuated light environment and pupose-built mixing apparatus allowed the simulation of natural conditions. Experiments examined each species by itself under mixed (10 min mixing interval) and "still" (48 h mixing interval) conditions in different light environments (high or low light attenuation). We observed the effects on growth rate, buoyancy or sinking and gross biochemistry of each species. Early results indicate that still environments with high light penetration provide no advantage to *A. circinalis* over mixed environments, however *Aulacoseira* sp. gains a growth advantage in mixed conditions by maintaining a higher position in the water column. We are currently defining the ecophysiological strategies of each species that allow them to dominate under specific environmental conditions, and testing our hypotheses in mixed culture competition experiments.

WATER DISCOLORATION AT EL-MEX BAY, WEST OF ALEXANDRIA (EGYPT) DURING 1992

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Mex Bay is a transitional marine system, which receives large amount of agricultural runoff (daily average $6.5x10^6m^3$), causing drastic environmental changes. The physical factors and the nutritional conditions; a daily external nutrient input and a quasi-permanent stable stratification create rich resource spectrum for algal growth; Five blooms causing water discoloration were observed in the period from April to September 1992. Eight different causative species were recorded for the five blooms, six of them are new as red tide species for Egyptian Mediterranean waters. The blooms resulted in an abnormal increase in biomass (Chl. a) and surface oxygen (up to a maximum of 160.3μg.l-1 Chl. a and 10.3 mlO2.l-1, 206.4% saturation, in April), and in a severe consumption of ammonia in preference to nitrate. Such blooms occurred at wide temperature and salinity range of 17.9-30.6°C and 23-38.5, respectively. The development of a pycnocline appears to have been a pre-requisite triggering factor. Nutrients seem to be un-limiting. The N/P ratio showed a decreasing trend with the development of the blooms, falling to a minimum on the peak days, recovering with the dissipation of the blooms. Dissipation of the blooms is assumed to be mainly due to grazing by fish filter feeders. Grazing by zooplankton seems negligible. The statistical analysis confirmed the significance of physical forcing (temperature, salinity and Sigma-t), on the growth of the red tides, but failed to define factors triggering their massive occurrence and drastic dissipation.

THE DEVELOPMENT, OCCURRENCE AND DISTRIBUTION OF TOXICITY IN EUKARYOTIC/PROKARYOTIC ASSEMBLAGES

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TEPS is multi-disciplinary project funded by the German Ministry for Education, Research, Science and Technology (BMBF TEPS 03F0161) to investigate the complex interaction between various pro- and eukaryotes that produce toxins in the marine ecosystems of the North and Baltic Seas. The TEPS program is divided into 3 phases. In the Characterisation Phase the molecular, morphological and biochemical features of selected toxic bacteria, algae and sponge species will be studied. In the Experimental Phase, the influence of biotic and abiotic factors on the expression of toxicity will be investigated. In the Developmental Phase, gene probes and novel means of measuring toxicity will be directed towards the production of a set of factors that can be used in an early warning system.

PSP TOXIN PRODUCTION OF URUGUYAN ISOLATES OF *GYMNODINIUM CATENATUM* AND *ALEXANDRIUM TAMARENSE* .

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It has been well documented that *Alexandrium tamarense* was responsible for paralytic shellfish poisoning (PSP) episodes along the coast of Uruguay during the spring of 1991, '92, '93, '95 and '96 while *Gymnodinium catenatum* has been determined to be the causative organism for PSP events which occurred during the summer-fall of 1992, '93, '94, '96 and '98. Cultures of these two dinoflagellates, established from the mouth of the Rio de la Plata, have been analyzed for saxitoxin and its derivatives by HPLC for the first time. These toxin profiles have been compared to shellfish extracts taken during 3 different toxic dinoflagellate booms. There is agood correlation between the toxin profiles of the shellfish collected in September 1991 and August 1993 and the cultured *Alexandrium* extracts whereas the best agreement between shellfish extracts from March of 1994 is with cultured *G. catenatum* extracts. The mouth of the Rio de la Plata is thus an area where two different dinoflagellates can cause PSP. Here we will explore some of the population dynamics of these two species to identify the extent to which blooms are distinct or overlap in time and space. We will also compare toxin profiles of Uruguyan cultures of *G. catenatum* to those of other *G. catenatum* isolates from other parts of the world.

EVOLUTION OF A *GYMNODINIUM BREVE* RED TIDE BLOOM ON THE WEST FLORIDA SHELF: RELATIONSHIP WITH ORGANIC NITROGEN AND PHOSPHOROUS.

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The Ecohab:Florida program conducts monthly quasi-synoptic cruises within an area on the West Florida Shelf that extends from Tampa Bay to Ft. Myers. Standard hydrographic measurements are made at 63 locations along three cross-shelf, one diagonal, and one along isobath transects. A bloom of *G. breve* was first detected off Ft. Meyers in November 1998 and persisted through January 1999 in this area. In February 1999, cell counts indicated that the bloom had moved north to the Tampa Bay/Sarasota region and the populations off Charlotte Harbor had disappeared. An examination of total dissolved phosphorous (TDP) and dissolved organic nitrogen (DON) for November through February revealed an estuarine signature for TDP, but not for DON. From May through October nutrient influx from offshore waters maintains a consistent, near-bottom chlorophyll maximum. Mixing of the water column in early fall due to seasonal overturn may resuspend DON from this deep maximum, increasing its presence in offshore waters, and potentially masking any estuarine signature. In January, however, DON concentrations were relatively higher inshore than on the outer shelf, coincident with entrapment by a coastal front of the nearshore waters containing the bloom. Throughout the duration of the bloom, local TDP and DON concentrations were not depleted, indicating either that *G. breve* is not using these nutrients in detectable concentrations or that influxes are offsetting any uptake by the bloom.

CHEMISTRY AND TOXICITY OF GYMNODIMINE AND ANALOGUES

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Gymnodimine was isolated from a *Gymnodinium* sp. for use in toxicological, chemical, and immunochemical studies. During the course of this work, two new hydroxylated isomers of gymnodimine, gymnodimine B and gymnodimine C, were isolated and their structures determined by spectroscopic methods. The LD50 of gymnodimine was determined in mice (i.p. injection, standard mouse bioassay procedure) and found to be 96 mg/kg. Gymnodimine was found to be unstable under some conditions, a factor that is likely to impact on its toxicology and on the development of instrumental and immunochemical assays for this toxin. The structures of the decomposition products, and their toxicities, are being investigated.

PRELIMINARY STUDIES ON *NOCTILUCA-PYRODINIUM* INTERACTION

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Pyrodinium bahamense var. *compressum* is the main organism causing toxic red tides in the Philippine waters. From 1987 when it first occurred in Manila Bay, the *Pyrodinium* recurred almost yearly up to 1998 except in 1997 which is an El Nino year.

Grazing is one of the factors considered to contribute in the decline of phytoplankton blooms. However, the role of grazers in the termination of *Pyrodinium bahamense* var. *compressum* bloom is completely unknown.

Preliminary in vitro feeding experiment showed that starved *N. scintillans* could feed on *Pyrodinium* cells. Filaments of mucus spread out from the cells of *Noctiluca* and entangled together to form a mucus web. *Noctiluca* fed on *Pyrodinium* cells trapped in the mucus and the aggregation caused the downward movement of *Noctiluca* which then discharged themselves from the web and float individually back to the surface.

The present study is a pioneering work to investigate the interactions of N. scintillans with *Pyrodinium* and to determine the impact of *Noctiluca* on *Pyrodinium* blooms.

ALGICIDAL MECHANISM OF *PSEUDOALTEROMONAS* SP. A25 CAPABLE OF KILLING *SKELETONEMA COSTATUM* WHICH INHIBITS THE GROWTH OF CULTURED *PORPHYRA* SPP.

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Algicidal bacteria belonging to genus *Pseudoalteromonas* or *Alteromonas* have been isolated from various coastal waters in Japan and Australia. However, little is known concerning the mechanisms of algal cell lysis by these bacteria at the molecular level. *Pseudoalteromonas* sp. A25 isolated from the water sample collected at the Ariake Sea, where red tide of diatoms have frequently occurred in winter seasons and have damaged to the production of *Porphyra* spp., exhibits strong algicidal activity. It could completely lyze a diatom *Skeletonema costatum* NIES-324 within 32 hours when inoculated at the density of 104cells/ml. Analysis of wild-type and a algicidal activity-lacking mutant of A25 by two-dimensional gel electrophoresis of cell proteins indicated that many proteins were induced in the stationary phase cells of wild-type. These proteins were not detected in the log phase cells of wild-type and also both in the log and stationary phase cells of the mutant. Algicidal activity against *S. costatum* was also detected only in the extract of wild-type cells collected in the stationary phase. These results strongly suggest the possibility that some of the enzymes induced in the stationary phase contribute to the lysis of *S. costatum* by *Pseudoalteromonas* sp. A25.

DYNAMIC *OF DINOPHYSIS ACUTA, D. ACUMINATA, D. TRIPOS* AND *GYMNODINIUM CATENATUM* DURING AN UPWELLING EVENT AT THE NW COAST OFF PORTUGAL

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The dynamics of *Dinophysis acuta, D. acuminata, D. tripos* and *Gymnodinium catenatum* was studied during the development of a weak to moderate upwelling event observed in a 9 days repeated coverage of a combined CTD/Phytoplankton section at 41;05'N off the coast of Portugal. All the species were mainly distributed in the surface wind driven layer within the region of the equatorward coastal jet. Wind induced mixing, offshore transport and vertical motions were reflected in the distribution and concentration of cells, depending on the relative position of each species in the water column before the northerly wind event. It was generally observed that wind relaxation was associated with blooming conditions while active winds gave rise to dispersion of cells and reduction of their number.

CAN WE PEACEFULLY LIVE TOGETHER WITH HARMFUL PHYTOPLANKTON? THE CASE OF THE GULF OF NAPLES

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A phytoplankton data series, regularly collected at a coastal monitoring station since 1984 and cyst surveys from sediments indicate that several potentially toxic species are present in the Gulf of Naples. These include species of the genus *Alexandrium* (e.g. *A. tamarense, A. minutum, A. andersoni), Dinophysis (D. sacculus, D. fortii, D. caudata, D. rotundata), Protoceratium reticulatum, Gymnodinium mikimotoi, G. cfr. catenatum*, *Chattonella subsalsa, Pseudo-nitzschia delicatissima*and *P. pseudodelicatissima.* Concentrations may be very high (up to $4x10^6$ cells l-1) in the case of *Pseudo-nitzschia* species at the long-term station. Other species, such as *Gymnodinium mikimotoi, P. reticulatum* and *C. subsalsa* are recorded sporadically and only from net samples. Still others, such as *Alexandrium andersonii* and *G. cfr. catenatum* have never been recorded in plankton samples but have recently been found during the course of a cyst survey in the area. This shows the advantage of integrating different sampling methods in order to have a more complete list of HAB species which are present in a given site. In the inner part of the Gulf there are several mussel cultivation farms, yet intoxication events attributable to harmful algae have never been reported in the area. We discuss the role of threshold concentrations of harmful species, intraspecific variability in toxicity, and oceanographic characteristics of the area among the possible reasons for the apparent lack of a negative impact on mussel cultivation and human health.

DETECTION OF DINOPHYSIS TOXIN-1 ALONG THE COAST OF MAINE

Steve L. Morton, Tod Leightfield, Mark Busman, Bennie R. Haynes, Debra L. Petitpain, Fran Van Dolah, and Peter D.R. Moeller

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Diarrhetic shellfish poisoning (DSP), a gastrointestinal disorder, is caused by the ingestion of shellfish contaminated with okadaic acid (OA) or one of the dinophysis toxins (DTX-1-3). The digestive gland of blue mussels (*Mytilus edulus*) collected from sites along Eastern Bay and Frenchman Bay, Maine displayed protein phosphatase inhibition activity. Subsequent analysis of these mussels by LC-MS/MS showed low amounts of DTX-1. To determine the source of this toxin, both phytoplankton samples and epiphytic samples were collected. Analysis of phytoplankton rich in *Dinophysis norvegica*, a known toxic species, showed no protein phosphatase inhibition activity, OA or DTX-1. However, analysis of macroalgal samples rich in the benthic dinoflagellate *Prorocentrum lima* displayed protein phosphatase inhibition activity and the production of DTX-1. Cultures of *P. lima* initiated from these macroalgal samples produced DTX-1 and small amounts of OA. This is the first study to link the toxic benthic dinoflagellate *Prorocentrum lima* with toxic shellfish in the Eastern United States.

PRODUCTION OF OKADAIC ACID AND DINOPHYSIS TOXINS BY DIFFERENT SPECIES OF *PROROCENTRUM*

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Okadaic acid and the dinophysis toxins (DTX-1-4) are primary agents responsible for diarrhetic shellfish poisoning (DSP) and maybe implicated as one of the many toxins resulting in ciguatera fish poisoning (CFP). Cultures of seven planktonic *Prorocentrum* species and nine benthic-epiphytic *Prorocentrum* species were analyzed for the production of cytotoxic compounds and protein phosphatase inhibition activity. Scanning electron microscopy positively identified all cultures. Cultures that showed phosphatase inhibition activity were analyzed for okadaic acid and the dinophysis toxins using LC-MS/MS. All seven species of planktonic *Prorocentrum (P. micans, P. triestinium, P. dentatum, P. minimum*, *P. compressum, P. balticum* and *P. areabium*) displayed no protein phosphatase activity. Of these species only a newly described species *P. areabianum* showed activity to the cytotoxic assay. Four of the nine benthic-epiphytic *Prorocentrum*species (*P. lima, P. hoffmannianum, P. belizeanum*, and *P. faustiae*) displayed protein phosphatase activity and only one (*P. mexicanum*) displayed cytotoxic activity. Subsequent analysis by LC-MS/MS shows the production of okadaic acid by *P. lima, P. hoffmannianum, P. belizeanum* and *P. faustiae*. However, only *P. lima* and *P. belizeanum* were shown to produce dinophysis toxins. Cultures of the benthic-epiphytic species *P. emarginantum, P. areanarium, P. elegans*, and *P. norrisianum* were found to be non-toxic.

MODIFIED PROTEIN PHOSPHATASE INHIBITION ASSAY FOR THE DETERMINATION OF TOTAL DSP IN CONTAMINATED MUSSELS

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The fluorescence protein phosphatase (PP-2A) inhibition assay was found to detect okadaic acid (OA) in mussels down to 1 μg/ 100 g of mussel tissue. It was more sensitive than the mouse bioassay (detection limit, 20 μg /100 g) or ELISA using the SCETI DSP check kit (detection limit, 10 μg / 100 g). A drawback of the PP-2A assay method has been its lack of sensitivity towards the esters of OA and DTX-1. This was addressed by including a hydrolysis step in the pretreatment of extracts which allows these derivatives to be converted to either okadaic acid or DTX-1 prior to the DSP assay. The method has been applied to the analysis of DSP in 21 samples of naturally contaminated mussels and the results from the PP-2A inhibition assay compared to those of HPLC. A good correlation was obtained for OA determined by the two methods in both unhydrolysed and hydrolysed samples. The new procedure will substantially reduce the incidence of false negatives in the DSP assay.

DIVERSITY, ABUNDANCE AND RISK FROM POTENTIALLY TOXIC BENTHIC DINOFLAGELLATES IN THE SYDNEY REGION

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In order to address the lack of information on the identities, diversity, and abundance of benthic dinoflagellates of Australian temperate marine habitats, we have conducted a survey of autotrophic and heterophic dinoflagellates in sediments of Botany Bay. This is the main port for Sydney, Australia, and is also a centre of shellfish aquaculture. In the period from November 1998-January 2000, 31 species of dinoflagellates in 9 genera were found, of which 14 are new records for Australia, and at least three appear to be organisms not previously described. The list to date includes to following species which have been reported to produce potentially harmful toxic compounds: *Coolia monotis* Meunier, 1919, *Amphidinium operculatum* Claparède and Lachmann, 1859, *Amphidinium carterae* Hurlburt, 1957, *Prorocentrum lima* (Ehrenberg, 1833) Dodge, 1975, and *Prorocentrum mexicanum* Tafall, 1942. The morphology of the organisms found will be described in detail, as well as their seasonal abundance. Planned further work includes the testing of cultures for toxicity.

DELINEATION OF DISTINCT ROUTES OF CA^{2+} INFLUX ASSOCIATED WITH BREVETOXIN- INDUCED EXCITOTOXICITY USING A FLUORESCENT IMAGING PLATE READER (FLIPR^{TM})

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Brevetoxins resemble domoic acid in that these phycotoxins produce an autocrine excitotoxicity in rat cerebellar granule neurons. The resultant excessive activation of glutamate receptors produces a dysregulation of neuronal Ca^{2+} homeostasis that ultimately leads to cell death. In the present studies we have used FLIPR^{TM} to monitor real-time alterations in cytoplasmic Ca^{2+} levels in primary cultures of cerebellar granule neurons. Cerebellar granule neurons (CGNs) grown in 96-well plates were used for intracellular Ca^{2+} $[Ca^{2+}]_i$ measurements at day 10-13 in culture. Cells were loaded with the fluorescent Ca^{2+} indicator, Fluo-3 AM to monitor dynamic changes in $[Ca^{2+}]_i$. Fluo-3AM is taken up by cells and entrapped intracellularly after hydrolysis to FLUO-3 by cytoplasmic esterases. FLIPR^{TM} possesses an automated 96- well pipettor which can be programmed to deliver fixed volumes of solutions simultaneously to all 96 culture wells from two separate 96-well source plates. Neurons were excited by the 488 nm line of the argon laser and Ca^{2+} -bound Fluo-3 emission in the 500-560 nm range was recorded with a CCD camera. Acute exposure to brevetoxins such as PbTx-1 produced a rapid and concentration dependent increase in intracellular $[Ca^{2+}]_i$. MK-801 pretreatment produced a marked reduction in the integrated Fluo-3 fluorescence response to PbTx-1. Similarly, tetanus toxin reduced the integrated Fluo-3 fluorescence response to PbTx-1 as a consequence of its ability to inhibit glutamate release. Additional pharmacological analyses of the PbTx-1 response revealed that L-type Ca^{2+} channels and the reverse mode of operation of the Na^{+}/ Ca^{2+} exchanger also contributed to the stimulated Ca^{2+} influx. These data document the utility of FLIPR^{TM} to delineate neuronal Ca^{2+} influx pathways associated with brevetoxin exposure.

PRODUCTION AND CHARACTERIZATION OF A MONOCLONAL ANTIBODY TO TYPE-2 BREVETOXINS.

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Brevetoxins (PbTxs), a class of marine lipid-soluble polyether compounds produced by the ichtyotoxic dinoflagellate *Gymnodinium breve* (*Ptychodiscus brevis*), are toxic for human both after direct exposure (sea-aerosol) or ingestion of toxic shellfish. Although numerous techniques for detection and quantitation of PbTxs are available or under development, including animal bioassays (mouse andfish), HPLC, molecular pharmacological assays using Voltage Sensitive Sodium Channel (VSSC) preparations, only the mouse bioassay is currently used to monitor the shellfish toxicity. Immunoassays (RIA and ELISA) using polyclonal antibodies have been documented since the pioneer study of Baden et al. (1984). However up to now monoclonal antibody (MAb) to PbTxs has not been produced. This work presents the first evidence of an unlimited source of standard material to design systems for detection, quantification, concentration and/or labeling of brevetoxins. Production technique and the full characterization of this Mab raised against type-2 brevetoxins are also described.

STRATEGY FOR THE DEVELOPPEMENT OF ANTIBODIES RAISED AGAINST CIGUATOXINS, THE USE OF BREVETOXINS AS MODEL FOR POLYETHER HYDROXYLATED COMPOUNDS.

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Ciguatoxins (CTXs) are highly toxic and poorly available low molecular weight hydroxylated polyether compounds synthesized by the benthic dinoflagellate *Gambierdiscus toxicus* Adachi and Fukuyo. They are transferred via the food marine web from the benthos to herbivorous and carnivorous fish and causes after ingestion of contaminated fish a human syndrome called ciguatera. For both immunization and assay purposes, it is necessary to covalently couple low molecular weight compounds (i.e. haptens) to immunogenic macromolecules, however difficulties arise from the lipophilic nature of CTXs and the absence of a functional group on these molecules. Using a brevetoxin congener (PbTx-3) as a model for hydroxylated polyether compounds, this work describes a new methodology for immunogen preparation dealing first with the micro-scale preparation of a toxin hemisuccinate derivative (PbTx-3 HS) and its subsequent conjugation to carrier proteins in a reversed micellar medium, with emphasis on the elucidation of optimal conditions for both synthetic steps. Second, considering previous demonstrations that the epitope density of the conjugates, the immunization schedule and the antibody repertoire can greatly influence the induction of specific antibodies (amount, class and affinity), two mice and a rabbit were immunized. The specificity and the affinity of antibodies raised against PbTx-3-BSA conjugates are presented and discussed. These results confirmed 1) the potential in preparing and characterizing immunogen with only 0.5 μmol of toxic compounds 2) the immunogenic properties of the conjugate 3) the applicability of this entire procedure to generate antibodies to ciguatoxins.

TOXIC CYANOBACTERIAL BLOOMS IN THE TAPA CURÁ RESERVOIR, NORTHEAST BRAZIL

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Annual rain deficit and lack of water renewal in 1998 linked to El Niño consequences seem to be responsible for the toxic cyanobacterial blooms of many water supply reservoirs of the Brazilian northeast. In Pernambuco State, there are growing records of neurotoxic *Cylindrospermopsis* blooms. The Tapacur· reservoir provides water for a population of 1 million inhabitants of the city of Recife, with total volume capacity of 94,200,000 m3. It is an hypereuthophic reservoir and cyanobacterial blooms are frequent around the year. This reservoir has been studied since May 1998 and the following variables were measured: cyanobacterial toxins by mouse bioassay, HPLC and imunoenzymatic assay (ELISA); phytoplankton population densities and composition according to Utermohl method, and limnological variables by standard techniques. Cyanobacteria were the dominant group of algae, representing at least 88% of the phytoplanktonic community. Cyanobacterial densities as high as 84,332 org/mL and chlorophyll values of 45.6 μg/L were observed on July 1998. At this period, *Cylindrospermopsis raciborskii* was the dominant species. On May 1999 a chlorophyll peak value of 88.7 μg/L was observed before the rainy season. Bloom extracts were tested by mouse bioassay and the symptoms observed were tremors, convulsion and respiratory arrest, with the time of animal death ranging from 2 to 5 minutes. Bloom samples collected from September 1998 to June 1999 showed constant neurotoxicity. A preliminary analysis indicated the presence of Paralytic Shellfish Poisons (PSP). On extracts of shrimp (*Macrobractum amazonicum*) collected from the reservoir, this analysis also indicated the presence of 24 μg STXeq/100g. Microcystin concentrations on phytoplanktonic cells were found to be low (mean value 0.44 ng/L). Despite of the presence of PSP toxins, Tapacur· waters continued to be used for supplying the inhabitants of Recife.

EVOLUTION OF A *GYMNODINIUM BREVE* RED TIDE BLOOM ON THE WEST FLORIDA SHELF: INORGANIC NUTRIENT SUFFICIENCY

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The Ecohab:Florida program conducts monthly synoptic cruises within an area on the West Florida Shelf that extends from Tampa Bay to Ft. Myers. Standard hydrographic measurements are made at 63 locations along three cross-shelf, one along shelf, and one diagonal transect. A bloom of *G. breve* was first detected off Ft. Myers in November 1998. The bloom persisted through February 1999, however, cell counts indicated that the bloom had moved north to the Tampa Bay/Sarasota area since January. Cell counts for the bloom ranged from 3000 to 1.123 x 106 cells l-1. Assuming a standard ratio of 106C:16N:1P (Redfield ratio) and a growth rate of 0.2 (cell doubling in about 5 days), then a range of 0.54-2.08 μmol N/day and 0.03-0.13 μmol P/day would be necessary to sustain the bloom. Examination of the inorganic N (NO3, NO2) available to the cells indicate insufficient N to support the bloom in every instance. In all but two of the stations NO3 was undetectable in the samples throughout the area and for the duration of the bloom. Available inorganic P was always sufficient to support the bloom, especially where high cell counts were found in November, December and January. The available inorganic P was likely sufficient to support this bloom event. It appears that organic N may be important in maintaining a G. breve bloom on the West Florida Shelf in the absence of sufficient inorganic N availability, as was found during the 1998-99 bloom event.

PARALYTIC SHELLFISH TOXINS IN *GYMNODINIUM CATENATUM* STRAINS FROM SIX COUNTRIES

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Blooms of *Gymnodinium catenatum* have been observed in Tasmanian waters since the mid-1980's and benthic cyst records suggest that it was not present prior to 1973. There is circumstantial evidence that it may have been introduced to Australia via ships ballast water. Disturbingly, *G. catenatum* has now been reported from several sites on mainland Australia, perhaps derived from Tasmanian populations. To examine these hypotheses we compared paralytic shellfish toxin (PST) profiles of a range of Australian (27 from 6 sites), Japanese (3), Portuguese (1), Spanish (5), Uruguayan (4) and Honk Kong (China) strains.

Duplicate cultures were analysed for PST by HPLC and the presence/absence of 13 characterised toxins was used as a basis to assess the similarity between strains. Two novel toxin-like compounds were identified in isolates from Australia, Uruguay and Hong Kong. These toxins, designated here as C5 and C6, were similar to PSTs in chromatographic behaviour and fluorescence properties and demonstrated activity in the saxiphilin binding assay, which has a highly specific affinity for PSTs. Australian strains contained either a broad combination of PSTs (C1-4, GTX1-5, dcGTX2-3, dcSTX, STX, C5 and C6, and deoxy-PSTs), or a combination of C5-6 and dcGTX2-3. Strains from the Derwent Estuary (Tasmania) and Port Lincoln (South Australia) exhibited very similar PST profiles, which were, in turn, similar to those from Hong Kong and Uruguay. Of the 12 strains isolated from Long Bay in Tasmania, a single strain contained a similar suite of toxins to Japanese isolates. The new C5 and C6 PST-like toxins, common to Australian, Hong Kong and Uruguay strains, show potential as a biochemical marker for between populations comparison of *G. catenatum* and may provide valuable insights into global dispersal and population relationships of this species.

INVOLVEMENT OF NADPH OXIDASE LIKE ENZYME IN THE PRODUCTION OF SUPEROXIDE ANION BY *CHATTONELLA MARINA*

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Chattonella marina, a raphidophycean flagellate, is one of the most noxious red tide phytoplankton and is highly toxic to fish, especially to yellowtail, *Seriola quinqueradiata*. Blooming of *Chattonella* has repeatedly caused severe damage to fish farming in Japan. One of characteristic features of this flagellate is the production of reactive oxygen species (ROS) such as superoxide anion (O^{2-}) and H_2O_2 under normal growth conditions. Since harmful effects of ROS have been well documented in various biological systems, ROS may be a responsible factor for fish mortalities by *Chattonella.* Although the detailed mechanism of ROS generation is still unclear, we have recently found that lectins such as concanavalin A (Con A), wheat germ agglutinin (WGA), and castor bean hemagglutinin (CBH) stimulate *C. marina* to generate increased amounts of O^{2-}, suggesting the presence of signal transduction pathway leading to O^{2-} generation similar to the oxidative burst in phagocytic leukocytes in mammals. In cell-free extracts prepared from *C. marina* cells, NAD(P)H-dependent O^{2-} generation was observed, and this response was blocked by diphenyleneiodonium (DPI), a potent inhibitor of mammalian NADPH oxidase. When the cell-free extract of C. marina was analyzed by immuno blotting using antibody raised against the human neutrophil cytochrome b558 large subunit (90 KDa), a major component of NADPH oxidase, several immunoreactive proteins were detected and one of them was approximately 110 kDa that was slightly larger than human cytochrome b558. These results suggest that *C. marina* have a plasma membrane enzyme system analogous to the neutrophil NADPH oxidase as a source of O^{2-} production.

THE DISTRIBUTION OF THE GENUS *PSEUDO-NITZSCHIA* OFF SOUTHERN BRAZIL AND RELATIONSHIPS WITH OCEANOGRAPHIC CONDITIONS

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The diatom genus *Pseudo-nitzschia* is widely distributed in shelf and slope waters off souhern Brazil (Lat 32-34oS; Long 50-530W). The concentration of *Pseudo-nitzschia* spp. cells was quantified in surveys from 1987 to 1990; highest values were observed in spring (mean 10^4 cells l-1; maximum 10^5 cells l-1), intermediate in summer (mean 10^3 cells l-1; maximum 10^4 cells l-1), and low in autumn and winter (mean 10^2 cells l-1; maximum 10^3 cells l-1). In estuarine waters of the Lagoa dos Patos (320S; 52015W) and adjacent coastal waters, high concentration of this diatom was observed in summer/autumn (1992-1995), with maxima of 10^5 cells l-1. The spatial and temporal distribution of the genus was associated to oceanographic features in the Southwest Atlantic Ocean, such as the freshwater outflow of Rmo de La Plata and Lagoa dos Patos and the presence of fronts due to mixing of waters of subantarctic and tropical origin. Six species were identified using light, scanning and transmission electron microscopy: *Nitzschia americana (=Pseudo-nitzschia americana), Pseudo-nitzschia fraudulenta, P. pungens, P. multiseries, P. pseudodelicatissima* and *P. australis*. The last four species are known as potentially toxic elsewhere, and five of the *Pseudo-nitzschia* species are also common in Argentinian continental shelf waters (36-48oS), indicating their wide spread distribution in the southwestern Atlantic Ocean.

REMARKABLE DIFFERENCE IN THE TOXICITY OF *ALEXANDRIUM CATENELLA* OCCURRED UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Alexandrium catenella has been known to bloom mostly in summer in Ofunato Bay, Japan. However, no significant toxin has been detected in shellfish during bloom of *A. catenella* in the bay. In 1998, bloom of *A. catenella* was observed in winter (November to December) as well as in summer (August to September). Considerable toxicity was detected in the shellfish during winter bloom of *A. catenella* whereas no significant toxicity was detected in the shellfish during summer bloom (August to September), though the cell density of the summer bloom was much higher than that of winter bloom, suggesting a large difference in toxin contents of the cells between summer and winter bloom. HPLC analysis revealed that the toxicity of natural cells from winter bloom was in the range of 60 – 170 fmol/cell, while that of summer bloom was below 5 fmol/cell. When the strains isolated from both summer and winter bloom were cultured under the same conditions, both strains showed similar toxin productivity with similar toxin profiles. The toxin contents of the cells of both strains increased with decrease of the temperature, showing 150 fmol/cell at 10 degrees C, the temperature at which the winter strain bloomed in the bay. These results show that difference in toxin contents of *A. catenella* cells under different environmental conditions is unexpectedly large. It seems to be difficult to assume the shellfish toxicity from the abundance of *A. catenella.*

PSEUDO-NITZSCHIA SPECIES IN THE FAR EASTERN SEAS OF RUSSIA

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The composition and distribution of *Pseudo-nitzschia* species in the coastal waters of the Far Eastern seas of Russia are not clear because *Pseudo-nitzschia* has often been identified only to the genus level. Electron microscopy observation revealed the presence of potentially toxic diatoms in the genus *Pseudo-nitzschia* on the Russian east coast. These species were *Pseudo-nitzscia pungens, P. multiseries* and *P. pseudodelicatissima. P. pungens* and *P. multiseries* are the most common and widely distributed species. They are abundant in the spring-summer period on the west coast of Bering sea and Kamchatka and in the coastal waters of Sakhalin island. The number of those species varied from dozen up to hundred of thousands cells per liter. The regular outbursts of *Pseudo-nitzschia multiseries/ P. pungens* (10^6cell l^{-1}) are recorded every year in June and September in the coastal waters of the Sea of Japan. *P. pseudodelicatissima* was found only in the coastal waters of the Sea of Japan and Sakhalin island. It is most common during mid- to late summer (10^4-10^5 cell l^{-1}). Dispersal mechanisms for *Pseudo-nitzschia* species are not known for the coastal waters of the Far Eastern seas of Russia. The wide distribution and ability to form blooms is probably explained by existing of resting stage in the life cycle of these species. According to the investigations of *P. pungens* in laboratory culture, these species can produce resting cells. It is supposed, that resting cells may be transported by currents or ballast water and survive until conditions are favorable for germination.

HUMAN TOXICOLOGY AND EPIDEMIOLOGY OF THE MARINE BLUE-GREEN ALGA *LYNGBYA MAJUSCULA*

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Lyngbya majuscula is a filamentous blue-green alga with worldwide distribution throughout tropical and subtropical regions. Since the first reports in the late 1950's in Hawaii and later in Okinawa, swimmers coming into contact with this cyanobacterium have contracted acute dermatitis. Strong anecdotal evidence suggests similar episodes around Fraser Island and other locations along the Queensland coast among recreational water users as well as commercial fishers. Of the over forty unique biologically active chemicals isolated from this species, three toxins, debromoaplysiatoxin, aplysiatoxin and lyngbyatoxin A have been found to be the major cause of dermatitis. These three toxins are all tumor promoters, binding to phorbol esters receptors leading to the activation of protein kinase C. Examination of the toxins present in the Australian blooms, and their biological activities will be made, as well as comparisons to other blooms of this species worldwide. A prospective cohort epidemiological study over three years will gather information on the location and type of water activity to assess the effect of algal blooms and toxin production on the health status of water users. It is envisaged that cohorts of volunteers, both recreational water users and commercial fishers, will be recruited at beach and other locations. Participants in the study will be asked to fill out a short questionnaire on location. Two to seven days later a follow-up telephone call will be placed and further questions on changes in health status 48 hours after water exposure asked. Assessment of change in health status will allow the first measurement of the number of people effected by this organism in Australia and estimates of the severity, spatial and temporal distribution of dangers and effects of length of time and use of clothing during water exposure. Correlation will be made with size of bloom, amount and type of toxins present and other environmental factors. Preliminary data will be presented at the conference.

TOXIC *NOSTOC* BLOOMS IN OUKAÏMEDEN RIVER (HIGH ATLAS OF MARRAKESH, MOROCCO)

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Health risks generated by cyanobacterial toxins in drinking and recreational water were clearly confirmed. During the survey programme (carried out since 1994) on distribution of toxic freshwater cyanobacteria in various water bodies including lake-reservoirs, ponds and rivers of Morocco, many cyanobacteria species dominated by *Microcytis* genus have been identified. Particular interest has been given to Nostoc species that was found dominant in Oukaïmeden River localised at 2600 m of altitude in high Atlas mountains of Marrakech. This benthic cyanobacteria appears covering the surface of rocks and sediment in a shallow zone of the river particularly along the river's banks where the current speed was less. The massive growth of Nostoc sp yearly occurred during the period June-September, where the average of water temperature was higher than 15 C. It seems that, an important input of organic matter in the river and particular water physico-chemical conditions were in favours of Nostoc blooms. The toxicity of the bloom material was confirmed both by mousse bioassay and ELISA. The major signs of mice poisoning were severe diarrhea, which appears 15 min after injection. The histopathological studies show a slight liver damage and remarkable destruction of intestinal mucosa consecutive of a collapse of villi architecture and a shedding of enterocyte cells. The detection and the content of total hepatotoxic microcystins was determined by using the Enzyme-linked immunosorbent assay (ELISA) which gave an amount of 12.8μg /g . Relationship between the content of microcystins and the growth stage of Nostoc were determined and discussed. The toxicity reported for this particular cyanobacterium strain was due to presence of hepatotoxic peptide toxins (microcystins) mixed with others unidentified toxins (likely neurotoxic compounds). The determination of toxigenic profile for this strain was not achieved. These preliminary results revealed potent sanitary risks generated by the existence of these toxic cyanobacteria water blooms in this important tourist site (High Atlas Mountains of Marrakesh region).

CONTRIBUTION TO SCREENING OF TOXIC MICROCYSTIS ISOLATED STRAINS FROM MOROCCO

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Cyanotoxins harmful effects on human health have been recognised particularly in countries where drinking water supplies contain toxic cyanobacteria. In Morocco, toxic cyanobacteria blooms are common in some water bodies used for recreational and/or drinking water reservoirs. According to a regulation concerning drinking water consummation as recently suggested by World Health Organisation (WHO), there is a need to establish a cyanotoxins national monitoring programme. During the first step of this investigation, many sites including rivers, ponds and reservoirs in central and south regions have been prospected in order to screen toxic cyanobacterial Moroccans strains. In this papers we present a results of toxicological study of the some forming-blooms cyanobacterial species: *Microcystis ichthyoblable* Kütz. isolated from eutrophic Oued El Mellah lake-reservoir (North of Casablanca city) and *Microcytis aeruginosa* Kütz isolated both from El Massira reservoir (south of Casablanca city) and Deroua fishponds system (Beni mellal city). These *Microcystis* strains cultured on Z8 medium under laboratory conditions presented a positive mousse bioassay with evident signs of hepatotoxicity. The content of "microcystins" was determined by the Enzyme-linked immunosorbent assay (ELISA). A comparison of these strains to *Microcystis aeruginosa* previously isolated from Lalla Takerkoust lake-reservoir, Southwest of Marrakech city (Oudra et al. 1998) showed a specific variability that will be discussed. This existence of such toxic forming-bloom cyanobacterium strains in these fresh water bodies leads us to think about a real establishment of a cyanotoxins-monitoring programme. Oudra B. et al.(1998) ˆ Occurrence of hepatotoxic *Microcystis aeruginosa* waterblooms in eutrophic moroccan lake-reservoir, 29-31. In "Harmful Algae", Reguera B., Blanco J.,Fernandez M.L. &Wyatt T. (Eds.) Xuntia de Galicia and IOC of UNESCO.

ON A *LINGULODINIUM POLYEDRA* BLOOM IN THE SETÚBAL BAY, PORTUGAL

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During 1996, the HAB monitoring program detected the presence of *L. polyedra* in the Setúbal Bay. One week later, a brownish discoloration of seawater was noticed and the species reached concentrations of 700,000 cells/l. The bloom lasted during ten days and the accompanying assemblage was dominated by *Prorocentrum micans*. An hydrographic/ phytoplankton cross-shelf section carried out in the Bay during the bloom period revealed conditions of stratification with a vertical gradient of 2.5 C between 10 and 30m depth. *L. polyedra* was distributed until 20m with the maximum over the thermocline, at 5m depth. After one week of bloom detection, the encystment process seemed to be initiated, since *L. polyedra* cysts were observed in the water samples representing about 0.1% of the species population. At the same time, sediment samples collected in a harbour south of Setúbal bay showed an increase in cysts of *L. polyedra* with apparently viable cell content. At this site, where a long-term cyst study is being conducted, the relative percentages of viable cysts of this species went on increasing until January 1997. Although *L. polyedra* has been related with yessotoxin production elsewhere, the present phenomena was not associated with any problems of toxicity.

PRODUCTION OF DOMOIC ACID BY *PSEUDO-NITZSCHIA PSEUDODELICATISSIMA* FROM THE NORTHERN GULF OF MEXICO

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Domoic acid (DA), a potent neurotoxin, is synthesized by certain members of the ubiquitous marine diatom genus, *Pseudo-nitzschia*. We have recently detected elevated concentrations of DA in phytoplankton field samples from the northern Gulf of Mexico. In searching for a possible source of the toxin, we have detected DA in cultures of *Pseudo-nitzschia pseudodelicatissima* isolated from this region and confirmed its presence using tandem mass spectrometry, preceded by liquid chromatographic separation of the toxin (LC-MS/MS). Unlike other toxic *Pseudo-nitzschia* species examined previously (e.g., *P. multiseries, P. australis*), cellular levels and production of DA in these *P. pseudodelicatissima* strains were highest in early exponential phase, while population growth rate was maximal. The maximum cellular DA activity measured in the culture by receptor binding assay was 34 fg DA equiv. cell-1. No net accumulation of toxin was evident in stationary phase, yet the distribution of DA shifted from predominantly intracellular to mostly extracellular during this stage of growth. This study unequivocally establishes *P. pseudodelicatissima* as a source of DA in the northern Gulf of Mexico. Moreover, our work suggests that rapidly growing, rather than nutritionally stressed, populations of this and possibly other *Pseudo-nitzschia* spp. may contain maximum cellular DA quotas, which is in contrast to the current paradigm of *Pseudo-nitzschia* toxin dynamics.

THE ROLE OF CELL OR COLONY SIZE AND TOXIN PRODUCTION IN PLANKTONIC CYANOBACTERIA AS STRATEGIES TO AVOID GRAZING.

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We evaluated the role of size selection and toxin production as strategies of cyanobacteria to avoid grazing by calanoid copepods. In the experiment I we investigated the effect of grazing of Notodiaptomus iheringi on the growth rate of 5 dominant species/group categories of a natural phytoplankton assemblage (*Anabaena* spp, *Cylindrospermopsis raciborskii*, single cells (3-8 μm), small colonies (<30 μm) and large colonies (>30μm)). The colonies and single cells were identified as *Microcystis* sp and *Chroococcus* sp. Experiment II aimed to estimate the grazing effect of *N. iheringi* on toxic and non toxic strains of *Microcystis aeruginosa* cultures. For the experiment I, the natural phytoplankton from Funil Reservoir (Brazil) was incubated with copepods (treatment) or without copepods (control). For experiment II, N. iheringi was fed with toxic or non-toxic unicellular *M. aeruginosa* or *Ankistrodesmus* sp previously grown as batch cultures. No copepods were added to the control bottles. The incubation time was 90 hours for both experiments and sampling for phytoplankton counting was done every 2 days. There were no microcystins detected (through HPLC) in the samples from Funil Reservoir, and the different size categories of cells/colonies of phytoplankton present in experiment I should therefore not have been toxic. The small colonies was the particle class mostly consumed by *N. iheringi* fed with a natural phytoplankton assemblage. In experiment II, ingestion rates of copepods fed with *Ankistrodesmus* sp was higher than the ones fed with any *M. aeruginosa*. No significant difference was found in the ingestion rates between the copepods incubated with toxic or non toxic *Microcystis*. These results suggest that *N. iheringi* did not graze efficiently on the small sized *Microcystis* cells (5 μm), no matter their toxicity. The results from the experiment I support this conclusion, since there was no significant grazing on single cells in the natural community. Also the larger particles (>30 μm) were not grazed efficiently during this experiment. Our results suggest that size selection may be more important than toxin production as an strategy against grazing, at least among *Microcystis* species.

DISINFECTION BY-PRODUCTS FORMATION POTENTIAL OF BLOOM-FORMING CYANOBACTERIA AND DIATOMS

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In many Korean lakes which are used as drinking water sources, algal bloom occurs every spring and summer season. A few cyanobacteral genus containing *Microcystis, Anabaena, Aphanizomenon* generally appear as the dominant components of summer blooms and diatoms containing *Synedra, Melosira, Cyclotella* as those of spring bloom and cause detrimental impacts to water usage such as clogging the sand filters, production of offensive odor and toxic compounds. Most of the water treatment plant in Korea are adopting the pre-chlorination process to treat algal bloom in the source water which probably cause production of disinfection by-products(DBPs). In this study, DBPs formation potentials of bloom-forming cyanobacteria and diatoms by chlorine dose and treatment time were investigated. DBPs analyzed in this study were trihalomethane, haloacetic acids, haloaceto nitrils, haloketones, chloral hydrate, and chloropicrine. For the *Microcystis* spp., release of microcystin during chlorination was also investigated. As chlorine dose was increased, concentration of DOC, THMs, and HAN was increased. Chloroform was found to be the major THM compound.

DIURNAL VERTICAL MIGRATION OF *COCHLODINIUM POLYKRIKOIDES* DURING A RED TIDE IN COASTAL WATERS OF NAMHAE ISLAND, KOREA.

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Since 1995, massive harmful blooms of the dinoflagellate *Cochlodinium polykrikoides* Margelef have been a annual feature along the southern coast of Korea in August through September, leading to serious impact on finfish farms covering huge area. As the vertical migration is important process to clarify the mechanism of red tide formation, the vertical distribution and variation of *C. polykrikoides* were investigated with time at a fixed-station off the coast of Namhae Island, Korea, enlightening the migration depth and velocity as well as the timing from downward to upward migration. The maximum cell density of *C. polykrikoides* was observed in the surface layer at 16:00 h. After then, the population of this species started to migrate downward and reached the bottom of 15 m by 19:00-20:00 h. The upward migration began at around 06:00 h and the population concentrated in the surface layer by 11:00 h. The speed of descending was faster than that of ascending due to gravity effect. The estimated velocity of vertical migration based on the depth of high cell density was > 3 m/h, which was fairly high compared to swimming speed of other dinoflagellates. Dividing cell was mainly observed in 04:00 to 08:00 h when the population of *C. polykrikoides* began to migrate upward from the bottom. During this high cell division, the cell density of chain groups of > 6 cells was much higher than that of < 5 cells. After 9:00 h, the density of long chain groups decreased with time, whereas that of short chain groups increased. This results suggest that these variations of each chain group with time (moreover the nocturnal cell division) can be a ecological strategy to get an impulse to migrate upward across the gravity barrier with long chain before sunrise and to obtain solar energy efficiently with short chain (high ratio of area to volume) after arrival to the surface layer.

THE SEXUAL LIFE CYCLE AND MATING SYSTEM OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM MINUTUM* DINOPHYCEAE) FROM THE PORT RIVER ESTUARY, SOUTH AUSTRALIA

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Alexandrium minutum (Halim) is the type species of the genus *Alexandrium.* It produces paralytic shellfish toxins and causes harmful algal blooms in many parts of the world including southern Australia, New Zealand, Spain, Japan, France and Portugal. The sexual life cycle of this species has not been fully elucidated although resting cyst germination was described by Bolch et al. (1991). We present here the complete life cycle of *A. minutum.* This was studied through detailed pair-wise crossing experiments with 16 strains isolated from the Port River Estuary, South Australia. Planozygotes with two longitudinal flagella were first observed less than 48 hours after crossing and the first hypnozygotes (resting cysts) observed after 5 days. Although previously thought to have a long requisite dormancy period of up to 6 months the dormancy requirement in our study was found to be only 4 weeks after which cells were able to germinate under optimal conditions (18 C, 12:12 light/dark cycle, nutrient replete media). The mating system of *A. minutum* was found to be complex, more similar to *A. tamarense* , than *A. catenella* which has a simple +/- mating system. Of a possible 78 pair combinations, 34 were found to produce resting cysts. The results show that there are at least 6 mating types or mating groups as well as some self compatible strains. There was also evidence of partial sexual compatibility between strains with either planozygote formation without resting cyst formation or formation of non-viable cysts. With the closure of this life cycle it is now possible to investigate the environmental cues for resting cyst formation (encystment) and resting cyst germination (excystment) and the role that these resting stages have in *A. minutum* bloom dynamics in controlled laboratory experiments.

HIGH DENSITY CULTIVATION OF *ALEXANDRIUM MINUTUM* (DINOPHYCEAE): EFFECTS ON GROWTH, LIFE HISTORY, FATTY ACIDS AND TOXIN PRODUCTION

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We present here a study of the feasibility of high density cultivation of toxic dinoflagellates. The benefits of producing high biomass of dinoflagellates include: 1) developing the capacity to produce high biomass for research and toxin standards, 2) investigating bioactive compound potential, 3) investigating physiology, life history, growth rate, fatty acid and toxin production using different culturing methods with implications for natural bloom populations and 4) culture technology development for production of fragile microalgae. *Alexandrium minutum* was used as a trial species for these methods of cultivation. Blooms of this paralytic shellfish toxin (PST) producing dinoflagellate are a significant problem in coastal and estuarine waters of both Australia and Europe. *A. minutum* is more robust than many other dinoflagellates, achieves high biomass in static culture, has a known toxin composition, its growth characteristics, mating system, and life cycle have already been determined in small scale laboratory culture and its bioactive compound potential is untapped. The life cycle of *A. minutum* incorporates both a vegetative and a sexual reproductive phase that results in the formation of resting cysts or hypnozygotes. Resting cyst formation is a valuable indicator of health, physiology and the interactions occurring between cells. This indicator was used in our assessment of high density culture of *A. minutum* along with growth and biomass measures, fatty acid and toxin composition. We first tested *A. minutum* in aerated and un-aerated medium scale batch culture before investigating growth potential in 40 cm glass aerated culture tubes which simulate a bubble column photobioreactor on a small scale and finally in a laboratory scale vertical alveolar panel photobioreactor.

ZOOSPORE PRODUCTION BY TWO TOXIC *PFIESTERIA* SPECIES AND THE BENIGN 'LOOKALIKE' SPECIES, *CRYPTOPERIDINIOPSIS*, GIVEN ALGAL PREY

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Zoospore production was experimentally examined during grazing on three species of algal prey (*Rhodomonas, Prorocentrum minimum*, or *Synechococcus* in single-species trials) by Neuse Estuary clonal isolates of *Pfiesteria piscicida*, the second known toxic *Pfiesteria* species ('B'), and the benign taxon *Cryptoperidiniopsis* nov. gen. (lack of bioactive compounds that cause fish stress, disease or death based on fish bioassays of multiple strains and species of this dinoflagellate from the Chesapeake Bay, the Albemarle-Pamlico, and three Florida estuaries). Prior to the experiments, strains of *P. piscicida* from the same clone had been maintained for 4 months in fish-killing mode (TOX – mildly toxic; fish death at 12- to 24-hour intervals with 5,000 cells/mL), temporarily nontoxic mode (NONTOX, on cryptomonad prey). An older clone of kleptochloroplastidic, 'never-toxic' strain (NEVTOX, grown on cryptomonad prey) that had lost its ichthyotoxic activity was also compared. TOX (highly toxic; fish death at 1- to 2-hour intervals with 800 cells/mL) and NONTOX strains of *Pfiesteria* B were compared as well. The *Cryptoperidiniopsis* strain was kleptochloroplastidic and had been maintained similarly as NONTOX *Pfiesteria* spp. Zoospore production by all species and strains was highest with cryptomonads among the three algal prey tested. TOX strains attained lower zoospore production on algal prey than NONTOX and NEVTOX strains. Weakly toxic *P. piscicida* showed higher zoospore production on algal prey than the highly toxic strain of *Pfiesteria* B; and both NONTOX *Pfiesteria* spp. achieved significantly higher zoospore production on cryptomonad prey than did *Cryptoperidiniopsis.* The data indicate that zoospore production on algal prey differs among *Pfiesteria* spp. depending on the strains' history of toxic activity, and that benign lookalike species such as *Cryptoperidiniopsis* should not be considered as comparable to *Pfiesteria* in their response to algal prey.

TUNA AQUACULTURE CAGES AND PHYTOPLANKTON CHLOROPHYLL BIOMASS RELATIONSHIPS : RANDOM OR REAL?

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Chlorophyll a analysis taken in the years 1995 to 1999 reveals anomalies concerning phytoplankton biomass in the vicinity of tuna farms at selected times of the year. In this preliminary study, we examined the relationship between 2 tuna farms (with controls), replicate 500m transects of surface water in vivo fluorescence and size fractionated chlorophyll a over 12 days in April 1999 in Boston Bay, Port Lincoln, South Australia. Pooled data from directional transects of in vivo fluorescence revealed some effect in the 0-50m range of the tuna cage with tuna cage 1 (TC1) being significantly different to the other three sites. Size fractions of chlorophyll a revealed a significant difference in the 5.0μm fraction for the combination TC1 and C1 indicating a strong affect of chlorophyll at the larger fraction of 5.0μm between TC1 and its control site C1 ($P=<0.01$). A significant difference was also recorded between the site areas TC1/C1 and TC2/C2 ($P=<0.01$). However no significant difference was found between TC2 and C2. The 0.45μm fraction was the smaller of the fractions, which showed that the major proportion of the phytoplankton resided in the nanoplankton.

INTERANNUAL STUDY OF THE INFLUENCE OF THE THERMOHALINE INESTABILITY IN THE BOOMS OF *DINOPHYSIS ACUMINATA*

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Main responsible of toxic episodes causing closure of mussel production areas in Galician Rías (NW Spain) is *Dinophysis acuminata*. The blooms of this species are a recurrent phenomenon with a clear seasonal pattern between spring and autumn. Due to the social and economical importance of the shellfish farmer sector in Galicia it would be very important a predictive tool for the incidence of this species.

In the developing of the *D. acuminata* populations it is important, in addition to the influence of the biogeochemical variables (nutrients, growth, grazing....), the influence of physical variables since they are planktonic organisms. In this sense, it has been demonstrated in culture the negative effect of the turbulence for the dinoflagellates and also it has been observed the relationship between *Dinophysis* spp and the stability.

An interannual study (1992-98), with a weekly sampling frecuency (in a station located in the middle part of the Ría de Pontevedra), showed the existence of a good relationship between the Brunt- Väisälä Period (T), and the blooms of *D. acuminata*. During the period 92-95, the average values and the oscilation of T was smaller and the proliferations of *D. acuminata* were more developed than in the 96-98 period. In the intermediate deep level (10-15 m.), the range of values of T is shorter than in the surface levels (0-5 and 5-10 m). In the three depth levels studied, the *D. acuminata* populations were small (no more than 1000 cells/liter) after winters with high values of T (more than 15 seconds). The inverse situation led to populations of this species up to one order of magnitude higher. This good relationship could be used as a predictor of the *D. acuminata* incidence in our area, whatever its causes would be.

NET GROWTH VERSUS TRANSPORT RATES OF HARMFUL MICROPLANKTON IN A COASTAL INLET AFFECTED BY WIND- DRIVEN UPWELLING (RIA DE AROUSA, NW SPAIN)

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An intensive hydrographic sampling (twice a week, 7 depth levels) was conducted in the middle Ría de Arousa (water depth 55 m) during the upwelling- favourable season (May- October) of 1989. This high sampling frequency allows to study the short- time- scale response of microplankton species to recurrent wind- driven upwelling events. Cell numbers above the standard baseline concentrations in the study area of the red forming species *Gyrodinium cf. aureolum* and *Heterosigma akashiwo* (eye catching red tide); the ichthyotoxic *species Dictyocha fibula* and *D. speculum* ($>10^4$ cells l- 1); the ASP species *Pseudo- nitzschia* spp. ($>10^6$ cells l- 1), the PSP species *Gymnodinium catenatum* ($>10^3$ cells l- 1) and the DSP species *Dinophysis acuta* and *D. acuminata* ($>10^3$ cells l- 1) were episodically recorded during the 6 months period. The contrasting causes of such accumulations net in situ, growth versus import from the adjacent shelf are quantitatively assessed by solving the classical 2- D non- stationary equation: where N is either cell or biovolume concentration, VX and VZ are the convective horizontal and vertical velocity fields, KZ is the coefficient of vertical turbulent diffusion and NEP is the net cell growth rate (production minus all losses). Cell concentration numbers have been obtained from counts on sedimentation chambers with the light microscope technique. Species biovolumes were estimated from direct size measurements and approximation to geometric forms. The values of VX, VZ and KZ have been estimated with a 2- D non- stationary box- model.

TOXIC TEMPERATE EPIPHYTIC DINOFLAGELLATES IN COASTAL LAGOONS OFF THE EAST COAST OF TASMANIA

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While toxigenic *Ostreopsis*, *Prorocentrum* and *Coolia* species are commonly recognized as tropical, epiphytic dinoflagellates, we report for the first time the occurance of this dinoflagellate assemblage, from temperate seagrass *Zostera mulleri* beds in sheltered lagoons of east coast Tasmania. Spatial distribution of these species was found to be limited by a preference for relatively high salinities, low turbidity and low nutrient levels. *Ostreopsis siamensis* was confined to the warmer, more saline months of the year. In culture this species grew (max. 0.53 div/day) at temperatures of 14-25 C and salinities of 30-45psu, however it was tolerant of temperatures as low as 10 C by the production of a newly recognized vegetative resting stage. These features suggest that the Tasmanian *O. siamensis* differs considerably from well studied tropical strains and may represent a new ecophenotype. *Coolia* cf. *monotis* grew at temperatures of 10-25 C and salinities of 15-45 psu. Both species in culture produced prolific mucus, and the possibility of this material exhibiting anti-foulant or anti-fungal activity is actively investigated. *Ostreopsis siamensis* tested positive for palytoxins (0.1 pg / cell) and the possibility of accumulation of these toxins in local shellfish is being investigated in view of a suspected case of human shellfish poisoning in Anson's Bay in early 1998.

DETECTION OF HAB SPECIES USING PCR-AMPLIFICATION TECHNIQUE AND SOLID-PHASE ELISA IMMUNOASSAY

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A method of PCR (polymerase chain reaction) and ELISA (enzyme-linked immunosorbent assay) immunoassay for the detection of the toxic *Alexandrium* species from cultured isolates and field samples of seawater is presented. The PCR-primers targeting the ITS-5.8S rDNA regions specific for the Alexandrium genus, amplified a fragment of approximately 286 bp (base pair) in the 5.8S ribosomal DNA (rDNA) and Internal Transcribed Spacer 1 and 2 (ITS1 and ITS2) regions from the *Alexandrium* isolates. The solid-fase elisa immunoassay involved application of a biotinylated-labeled primer targeting the ITS-5.8S rDNA regions; the PCR-amplified products, containing the digoxigenin-11-deoxiuracil triphosphate nucleotide (dig-dUTP), were captured on the streptavidin-coated microplate. The captured molecule were hybridized to an anti-digoxigenin antibody conjugated with alkaline phosphatase activity, which served to develop an enzyme-driven colourimetric reaction. The results indicated that the genus-specific biotinylated primer recognized the ITS-5.8S rDNA target sequences of Alexandrium cultured isolates by capturing streptavidin molecules-coated microplate, but did not target rDNA from other closely related groups of microalgae or marine phytoplankton populations of seawater samples, in which the *Alexandrium* species were absent; furthermore, the number of *Alexandrium* cells in the samples resulted in a proportional appearance of colour generated by the phosphatase activity in the presence of a chromogenic substrate and measured in a plate reader. The reproducibility and variability of this method were also tested. Thus, the immuno-PCR and ELISA assay is a useful technique to detect the presence of the target microalgal species in cultured and field samples; this method seems to be faster and simple, compared with other identification methods currently in use.

MOLECULAR CHARACTERIZATION OF MEDITERRANEAN ISOLATES OF THE HAB DINOFLAGELLATE *ALEXANDRIUM TAYLORI:* A PRELIMINARY INTRA- AND INTERSPECIES ANALYSIS

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High-biomass blooms of *A. taylori* have recently been spreading over new Mediterranean areas, with evident adverse effects on the marine ecosystem. Green-brown tides due to the outbreaks of this dinoflagellate are also causing loss of recreational value of coastal regions and considerable economic setbacks to the local tourist industry. In 1996-1999, a number of sites have been affected by summer blooms of *A. taylori*: Ionian coast of Sicily, Eolian Islands/Vulcano, Balearic Islands, Catalan coast of Spain, with maximum cell densities ($1.2 \cdot 10^7$ cells L-1) observed in La Fosca, St. Pol, Paguera and Vulcano together with water discoloration. Clonal cultures (AT-4 and AV-8 strains) established from Italian and Spanish seawater samples were used for the 5.8S rDNA gene and ITS region sequence analysis in order to initially test the possible intraspecific genetic variability of geographically distinct populations and develop further molecular markers for HAB, Mediterranean key-species. From the sequence analysis, the Italian and Spanish strains of *A. taylori* proved to be closely related to each other, but very distinct from species with similar morphological features, such as *A. margalefi* (subgenus *Gessnerium*) recently found in the Tyrrhenian Sea. Molecular characterization of other populations of *A. taylori*, as well as other *Alexandrium* species from the Mediterranean area, are in progress to better define genetic divergences and species boundaries.

CO-OCCURRENCE OF MICROCYSTINS AND SAXITOXINS IN MONTARGIL RESERVOIR, PORTUGAL.

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Portuguese National Monitoring Program of Cyanobacteria and Microcystins in drinking and recreational water reservoirs was implemented in 1996 by a working group co-ordinated by the Portuguese General Directorate for Health. In order to point out the most problematic regions, regular phytoplankton quantifications were carried out in 1996 in several Portuguese freshwater reservoirs. In Montargil reservoir (39;N, 8;W) a shift from the dominant group of Chlorophyceae to Cyanophyceae was observed during the warm period, indicating eutrophication of the lake, and an intensive bloom of phytoplankton was detected in May, with algal material accumulating on water surface. The algal community was strongly dominated by cyanobacteria and the extracts of the samples collected during the bloom period revealed unusual high toxicity by mouse bioassay. In order to distinguish, characterize and identify the organisms and the toxins responsible for the observed toxicity, strains of the two predominant cyanobacteria, *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* were isolated from the natural samples and established in permanent culture. Here, we report the presence of both PSP toxins and microcystins, produced by different cultured cyanobacteria strains isolated from the same bloom. The co-occurrence of both types of toxins in freshwater environments is probably a frequent situation and demands careful attention in assessing risk for human health.

ALEXANDRIUM AND *PROROCENTRUM* TOXINS: EFFECT ON CASPASE ACTIVITIES IN PC12 CELLS AND PRIMARY NEURONAL CELLS

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The effects of culture supernatants from different dinoflagellate (*Alexandrium* and *Prorocentrum*) clones on the activities of caspases involved in apoptotic pathway were determined. Exposure of rat pheochromocytoma PC12 cells and rat primary neuronal cells to toxic supernatants from *A. lusitanicum* ME-091, *A. lusitanicum* K2, *A. tamarense* 1M, and *A. tamarense* ccmp 115 for 1 to 3 days resulted in a time-dependend increase (of up to 188%) in the specific activities of caspases 1 (ICE), 3 (CPP32) and 6 (Mch2). The extent and the time point of the maximal increases in caspase activities varied between the different dinoflagellate clones. A significant increase in the specific activities of caspases 1, 3 and 6 was also observed after treatment of neurons with the supernatants of P. lima ME-130 clones K1 and K2. The maximal increases in caspase activities in PC12 cells (CPP32, 364%; and Mch2, 166%) and in neurons (CPP32, 162%) were observed 24 h after treatment with the *P. lima* ME-130 K1 supernatant. Among the isolated dinoflagellate toxins tested, saxitoxin caused a strong increase (up to 205%) in all three caspase activities in neuronal cells but not in PC12 cells. Tetrodotoxin had, if at all, only in a small effect on caspase activities in these cells. The protein phosphatase inhibitor okadaic acid caused a time-dependent increase in caspase activities in PC12 cells. A much higher effect was observed in neuronal cells. These results indicate that induction of apoptosis in PC12 cells and primary neuronal cells by toxic dinoflagellate supernatants and isolated dinoflagellate toxins may be mediated by an activation of intracellular caspase activities. Supported by the German BMBF, TEPS Project

RAW CULTURES – A USEFUL TOOL IN SEARCH FOR ORGANISMS PRODUCING RESTING-STAGES

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Raw cultures were used successfully in experiments aiming to survey the northern Swedish west coast for dinoflagellate cysts as well as in a pilot study to investigate the presence of phytoplankton resting stages in ballast water and sediment.Untreated aliquots of sediment or concentrated (by filtration) ballastwater was added to culturing flasks containing filtered seawater and put inculturing chamber to allow germination of resting stages. The resultingmixed cultures of vegetative stages were studied for a period of two weeksand documented directly in the flasks using an inverted microscope. For proper identification of dinoflagellates from sediment samples additional cultures of concentrated cyst fractions were used. This simple and unselective germination of resting stages proved to be a very useful complement to a "traditional" dinoflagellate cyst survey or a survey for resting stages in ballast sediment or water. Many organisms appeared in the cultures that were not found as resting stages, probably due to that their resting stages either were few, small and inconspicuous or of unknown appearance. Cultures of sediments from the Swedish west coast contained copepods, rotifers, ciliates (at least 50 types), dinoflagellates (at least 47 species), diatoms, cyanobacteria, haptophytes, cryptophytes, euglenophytes and chlorophytes. Cultures of ballast water and sediment contained mainly cosmopolitan diatom species.The use of raw cultures could increase the speed and economy in surveys ofresting stage-producing toxic species in sediments in the future if used incombination with modern recognition methods that are being developed forrecognition of toxic species in plankton samples.

INHIBITION OF HUMAN RECOMBINANT GLUTATHIONE S-TRANSFERASE ACTIVITY BY CYANOBACTERIAL LIPOPOLYSACCHARIDES ˆ SUPPORTING THE HYPOTHESIS OF THE INFLUENCE OF LIPOPOLYSACCHARIDE ON THE TOXICITY OF MICROCYSTIN-LR

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A number of human adverse health effects have been recorded due to effects of microcystins including the deaths of over 60 haemodialysis patients at a clinic in Brazil. In this study it has been shown that microcystin-LR (MC-LR), one of the most common cyanobacterial hepatotoxins was conjugated to glutathione by recombinant human glutathione S-transferase (GST) (E.C. 2.5.1.18) isoenzymes A1-1, P1-1 and M1-1. The kinetic reaction of the conjugation was time- and dose-dependent. The glutathione conjugate is suggested to be the first step in the detoxication of MC-LR. It is well known that Gram-negative bacteria including cyanobacteria include lipopolysaccharide (LPS) endotoxins in their outer cell layers. Bacterial LPS are recognised to be involved in septic shock syndrome, which may potentiate toxicant-induced liver injury. In rats, LPS significantly inhibited the expression of microsomal epoxide hydrolase and GST genes. In the presence of cyanobacterial crude cell extracts and purified cyanobacterial LPS from various sources (axenic *Microcystis aeruginosa* CYA43 and two natural blooms of *Microcystis* sp. and *Gloeotrichia* sp.) the activity of GST isoenzymes towards CDNB was reduced in a dose-dependent manner. The ability of the isoenzymes to conjugate to MC-LR was also suppressed significantly in the presence of the various LPS. For human risk assessment future analysis of cyanobacterial toxins including endotoxins might be necessary, because these results strengthen the hypothesis that the toxicity of MC-LR is increased in the presence of LPS by the inhibition of the GST detoxification enzyme system.

THE EFFECTS OF IRON LIMITATION ON GROWTH AND PHYSIOLOGY OF THE COASTAL MARINE RAPHIDOPHYTE, *HETEROSIGMA*

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Increasing occurrence of bloom outbreaks of the marine flagellate *Heterosigma* have been reported over the past few decades along coastal regions around the world. Iron is an important element, often implicated in controlling bloom initiation and maintenance. As a coastal bloom former this species has an unusually high requirement for iron, yet blooms in an environment where iron may be in a form not be readily available to the cell. Ultimately, the lack of available iron may limit the extent and duration of the bloom.

An understanding of the cellular uptake mechanisms is needed to understand the importance of iron in the ecology of *Heterosigma* sp. We have examined a collection of toxic and non-toxic isolates of *Heterosigma akishiwo* (primarily from the coastal waters of British Columbia) for their ability to scavenge iron from different sources. In general, *Heterosigma* cells have demonstrated a high iron requirement, much higher than *Prorocentrum* sp., for example, yet can adjust their iron quota to match the available iron. This allows for growth over a broad range of iron levels. This observation has prompted the examination of key physiological processes to elucidate potential alterations in cellular form and/or function in response to iron limitation. Physiological responses that are characteristic of an induced iron stress, such as chlorosis, the depletion of cytochrome f pool, and a change in the ratio of soluble ferridoxin to flavodoxin were apparent. The importance of characterizing these modifications is two-fold. Firstly, they may provide evidence of cellular adaptations to variable iron conditions, and secondly, serve as an essential diagnostic tool in field studies as biomarkers of iron stress.

OBSERVATIONS ON THE BLOOM DYNAMICS OF OKADAIC ACID PRODUCING DINOPHYSIS SPECIES AND THE CONSEQUENT CONTAMINATION AND DEPURATION OF SHELLFISH IN THE SOUTHERN BENGUELA UPWELLING SYSTEM

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Diarrhetic Shellfish Poisoning (DSP) was identified on the South African coast for the first time in 1991, and attributed to the dinoflagellate *Dinophysis acuminata*. Subsequent monitoring has revealed that DSP is commonplace on both the West and South coasts and although D. acuminata is the most common species several other *Dinophysis* species known to cause DSP have been recognised as a component of the phytoplankton. These include *D. fortii, D. hastata, D. tripos* and *D. rotundata*. Time series of *Dinophysis* concentrations on the West and South coasts has revealed their intermittent presence throughout the upwelling months, occurring in relatively low concentrations, as a component of multispecific populations. Despite considerable interannual variation in both the species present and in the observed cell densities, cell concentrations tend to peak in the latter half of each upwelling season. At the event scale the incidence of *Dinophysis* is associated with the onshore and offshore movement of dinoflagellate-dominated frontal blooms contributing to high within-season variability. Within the frontal blooms *Dinophysis* species are often associated with species responsible for red tide. Here the specific growth rates of Dinophysis cells are low and *Dinophysis* cells display limited vertical migration in comparison to other members of the red tide assemblage. In the stratified waters offshore of the front *Dinophysis* cells are prevalent subsurface and often associated with a heterotrophic community. Time series analysis of DSP toxins enabled their periodicity to be established in relation to meteorological and hydrographic events. Estimation of water column toxin concentration, derived from cell concentration and cellular toxicity, provided an opportunity to describe a functional relationship between toxin concentration and bivalve toxicity. The time lag between observing toxic phytoplankton in the water and elevated shellfish toxicity was brief, as was the lag between the resumption of upwelling and declining shellfish toxicity. Nevertheless calculated and observed rates of toxin accumulation and depuration in bivalve populations showed that the regulatory level is exceeded for a considerable part of the year.

BROWN TIDES IN AN EMBAYMENT ON THE SOUTH AFICAN COAST: CAUSES AND CONSEQUENCES

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In January 1997 an oyster grower in Saldanha Bay complained of discoloured water and reduced oyster growth rates. Inspection of water samples revealed a bloom of minute, coccoid, non-motile cells. The following year the bloom again appeared, and on that occasion the entire Saldanha Bay and Langebaan lagoon were discoloured a very unusual and distinct golden brown. This bloom persisted for several weeks attaining cell concentrations in excess of 3x109 cells.l-1 and chlorophyll a concentrations exceeded 40 mg.m-3. Transmission electron microscope, high performance liquid chromatography and immunological studies have confirmed the identity of this picoplankter as Aureococcus anophagefferens. This species was first described in 1988 after very similar blooms, aptly named "brown tides", impacted coastal embayments along the mid-Atlantic coast of the United States. The incidence of these blooms on the South African coast represents the only other record of major blooms outside the United States. Locally, the bloom impacted dramatically on mariculture activities within the region causing growth arrest in both oysters and mussels. As a consequence oyster sales in the region during 1999, following reappearance of the bloom, have declined to levels which now threaten the continuation of oyster farming in the region. Feeding experiments conducted with oysters confirm that feeding on the *Aureococccus* bloom, particularly by large oysters, is inefficient. Nitrate uptake experiments have indicated that NH4 is utilised more efficiently than NO3 and with a greater capacity. Urea is also taken up by *Aureococcus,* but in a complicated pattern. The fact that *Aureococcus* is physiologically better equipped to exploit reduced nitrogen confers a competitive advantage under conditions of reduced flushing and restricted input of NO3. Local recycling processes, within shellfish farms, such as excretion and decomposition, increase the availability of reduced nitrogen and serve to favour bloom development. The added advantage of a size-based refuge from heavy grazing pressure and minimal sinking losses act in concert to extend the duration of *Aureococcus* blooms.

DINOFLAGELLATE *ALEXANDRIUM* IN THE UPPER GULF OF THAILAND

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Two species of dinoflagellate genus *Alexandrium* were isolated from 8 different locations around the upper Gulf of Thailand apart from 17 sampling sites. *A. tamarense* was widely distributed in the salinity ranged from 19 to 40 psu in aquaculture ponds in five provinces as well as from Rayong river mouth. *A. minutum* was found in the lower salinity level of 15 psu in Chao Praya river mouth. Clonal culture of these *Alexandrium* exhibit the specific growth rate of 0.40 to 0.65 per day. Only extracts from 4 clonal cultures of *A. minutum* were toxic. The toxicity varied from 1.12 x 10-4 to 1.53 x 10-3 MU/cell. Toxin profile composed of GTX1-4 with the GTX1 as the dominant component. However, the density of *A. minutum* found in the sampling site was extremely low, 24 cells/l, to threaten the aquatic lives and human health in the area. Mouse-bioassay method as well as reverse-phase HPLC toxin analysis reviewed that *A. tamarense* found during this study was not harmful, since the extracts from 7 strains collected around the upper Gulf of Thailand was not toxic and did not contain any GTXs and STXs toxin.

A RECEPTOR BINDING ASSAY FOR PSP TOXINS: RECENT ADVANCES AND APPLICATIONS

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We recently described a high throughput receptor binding assay for paralytic shellfish poisoning (PSP) toxins and its use for detecting toxic activity in extracts of various shellfish species and *Alexandrium* spp. cultures. The efficiency of this assay has been increased dramatically through reformatting to accommodate microplate scintillation technology, yielding a turn around time of 4 hours for a 96-well plate. In collaboration with colleagues at NEN Life Science Products(Boston, MA, USA), we have also validated the use of ^{3}H-tetrodotoxin (TTX) as an alternative radioisotope to the conventionally employed ^{3}H-saxitoxin (STX). Either 3H-TTX or 3H-STX can be used interchangeably with no compromise in assay performance, which allows for greater flexibility in response to restricted isotope availability. Efforts are now being focused on demonstrating the range of applications for which this receptor assay can provide data comparable to the more time consuming, technically demanding HPLC analysis of PSP toxins. To date, we have compared the results of both methods for a variety of sample matrices, including different genera of PSP toxin producing dinoflagellates(e.g., *Alexandrium fundyense*, r2=0.8727, n=17), size-fractioned field samples of *Alexandrium* spp. (20-64 μm; r2=0.9998, n=7) as well as its associated zooplankton grazer community (200-500 μm; r2=0.4715, n=10), and contaminated human fluids (r2=0.9661, n=7) from a PSP outbreak. Our findings show that the saxitoxin equivalent values generated by receptor assay for all but the zooplankton samples are in very close quantitative agreement with those produced by HPLC, yet the former can be obtained in considerably less time. While the PSP receptor binding assay does not provide information on toxin composition, it does represent an effective means of rapidly assessing toxicity in sample matrices from the laboratory and the field, as well as identifying samples for more detailed analysis by HPLC.

HARMFUL MICROALGAE FROM THE GULF OF THAILAND

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Seventeen species of a potential harmful microalgae were encountered during a survey of phytoplankton diversity project from the Gulf of Thailand. Fifteen are dinoflagellate and the rest are diatom. Seven species (*Rhizosolenia styliformis, Trichodesmium erythraeum, Ceratium furca, C. trichoceros, Gymnodinium sanguineum, Noctiluca scintillans* and *Prorocentrum micans*) can be the causative organisms for red tide phenomena. Four (*Alexandrium minutum, A. tamarense, A. tamiyavanichi* and *Gymnodinium cf. catenatum* are PSP-causing, three (*Dinophysis caudata, D. miles* and D. mitra) are DSP-causing and the rest (*Amphidinium caterae, A. klebsii* and *Prorocentrum lima*) are ciguatera-causing organisms. Even the red tide is sometimes occurred in the Gulf of Thailand but no incident of toxic shellfish poisoning was reported since 1983.

DETERMINATION OF MICROCYSTIN-LR BY SOLID PHASE MICROEXTRACTION-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Kai-fai Poon, Michael Hon-wah Lam & Paul K.S. Lam

A fast, simple and sensitive method for the determination of cyanobacterial hepatotoxin, microcystin-LR (MC-LR) , was developed using solid phase microextraction coupled to high performance liquid chromatography (SPME-HPLC). Extraction and desorption conditions, such as pH and ionic strength of the sample medium as well as sampling and desorption duration, of the SPME process were optimized. Partition coefficient for the distribution of MC-LR between the PDMS/DVB-SPME stationary phase and the aqueous phase was estimated to be 5.4+ 0.18 x 103. Relative repeatability of 6.4% (n=10, P>0.05) and detection limit of 10 ng/ml (n=8, P>0.05) were achieved.

COASTAL DOWNWELLING AND DINOFLAGELLATE DOMINANCE IN THE RIAS OF GALICIA

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In the recent past it has been suggested that downwelling is the oceanographic process responsible for the initiation of harmful dinoflagellate blooms in the Rias Baixas, Northwest Spain. The studies that supported this hypothesis came from short-time observations during red tide events in the region. It appears necessary to confirm or reject the hypothesis using a more intensive sampling frequency in the area. We present here the results obtained from a twice weekly sampling conducted in a station located in the middle of Ria de Vigo over an annual cycle. The Ria de Vigo is located in an area subject to seasonal upwelling and it has been found that the seasonal evolution of total microplankton abundance follows that of diatom abundance except during strong downwelling in autumn. During downwelling diatoms disappeared from the water column and motile forms such flagellates and large dinoflagellates almost exclusively dominated the microplankton population. The results therefore confirm the previous hypothesis that downwelling events are necessary to remove diatoms from the surface layer, which creates a niche for motile forms, which can grow if environmental conditions (mainly nutrients and light) are favourable. It may be concluded that downwelling is the general mechanism that promotes dinoflagellate red tides in the region as opposite to the dualism upwelling-diatom blooms. A similar mechanism could operate in other semi-enclosed basins under the influence of upwelling-downwelling cycles.

PHYSIOLOGY AND BEHAVIOR OF THE TOXIC DINOFLAGELLATE, *ALEXANDRIUM TAMARENSE,* FROM CASCO BAY, MAINE (U.S.A.) DURING NITRATE LIMITATION

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Significant variability has been observed among strains of *Alexandrium* from the northeastern US and Canada with respect to morphology and toxin composition. Our objective was to study variability in acquisition of deep nutrients, specifically nitrate, via diel vertical migration (DVM), comparing the behavior and physiology of *Alexandrium* isolates from Casco Bay, (Maine) and the Gulf of St. Lawrence (Canada).
Previous work has established that an *Alexandrium* isolate from the Gulf of St. Lawrence can acquire nitrate in the dark during nocturnal descent to the nitracline. This migration behavior is thought to be a survival strategy of dinoflagellates, enabling them to persist longer in stratified coastal environments. The presence or absence of DVM behavior was examined in isolates of *Alexandrium* from Casco Bay using thermally stratified laboratory water columns (mesocosms). Temperature in the mesocosms ranged from 17 C at the surface to 7 C at the bottom with a surface irradiance of 250 μmol quanta m-2s-1 (14h light:10h dark cycle), identical to the previous study using a Gulf of St. Lawrence isolate. Like the St. Lawrence strain, the cells maintained a thin surface layer, depleting nitrate (initially 50μM) from the top 20cm within a few days. Unlike the St. Lawrence strain, which acquired nitrate through nocturnal migration to the nitracline, the Casco Bay cells did not deplete the tank of nitrate and showed signs of progressive N limitation. Calculations show that nocturnal nitrate uptake in the mesocosm after Day 29 was inadequate to sustain a growth rate of 0.3 day-1 as determined from nitrate replete cultures. These and other studies on nitrate acquisition have significant bearing on observations of *Alexandrium* in the field , notably their ability to persist in nutrient deplete surface waters. In the Casco Bay region cell densities are often quite low during blooms, consistent with an inability to acquire deep nitrogen.

COMBINED EFFECTS OF LIGHT, SALINITY AND INTENSITY OF ILLUMINATION ON THE GROWTH OF *HETEROSIGMA AKASHIWO, ALEXANDRIUM TAMARENSE* AND *SKELETONEMA COSTATUM*

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Combined effects of salinity, temperature, and light intensity of illumination on the growth of three red tide species: the raphidophytes *Heterosigma akashiwo* (fish-killing species), the dinofllagelate *Alexandrium tamarense* (PSP producer) and the diatom *Skeletonema costatum*, were examined in three laboratory experiments. The experimental ranges of salinity (10 to 35S), temperature (12 to 32 C), and light intensity (0.05 to 1.6 x 10^{16} quanta.sec-1 cm-2) were comparable to those encountered in the Pearl River Estuary and the Hong Kong waters. The experimental setup followed a 3-factor design, using 4 temperature levels (12, 19, 25, 32 C), 5 salinity levels (10, 18, 25, 30, 35S) and 4 light intensities of illumination (0.05, 0.1, 0.3, 1.6 x 10^{16} quanta sec-1 cm-2) for a total of 80 treatments per species. The results showed that, in terms of affecting algal growth, temperature interacted with illumination, so as temperature and salinity, but illumination did not interact with salinity. The optimal growth condition for *H. akashiwo* was of 25 C, 10-35S, 1.6 x 10^{16} quanta sec-1 cm-2; for *A. tamarense* was of 19 C, 30S, 1.6 x 10^{16} quanta sec-1 cm-2; and for *S. costatum* was of 25 C, 18-35S, 0.3-1.6 x 10^{16} quanta sec-1 cm-2. These results indicated that in the Hong Kong waters, *H. akashiwo* and *S. costum* could divide quickly and likely bloom from late spring through fall when temperature and illumination are at high. Both species shall be able to bloom in both the western water (the Pearl River Delta) and the eastern water of Hong Kong (Oceanic water), due to their high resistance to relatively low salinity. *A. tamarense*, however, is more likely to form red tide in either early spring or late autumn only in the Eastern water of Hong Kong (Oceanic condition). A brief review on the red-tide events reported in Hong Kong water for the last 15 years agrees well with our experimental results. We, therefor, concluded that well-designed indoor experiments can help us explain red-tide events and may serve as powerful tool for developing predictive or alerting models of red tide.

BLOOMS OF *MICROCYSTIS AERUGINOSA* IN A FRENCH RESERVOIR : TOXIN PRODUCTION AND BIOGEOCHEMICAL APPROACH

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Villerest Lake (France) is a reservoir on the Loire River. This lake is characterized by repetition of annual blooms of cyanobacteria *Microcystis aeruginosa*. Since more than 10 years, blooms appeared during the estival period, and for 1 to more than 3 months, perturbing the tourist development of villages near the lake. I order to estimate the toxicity of these blooms, the production of microcystins was studied here, by phosphatase tests and HPLC detections, from August 1998 to October 1999. This was observed parallely to biogeochemical parameter variations in the water column. Positive results have been obtained during water blooms by phosphatase tests and HPLC analyses, revealing microcystin production in the lake. Cyanobacteria development coincides with the observation of the lowest NO3- concentrations and an evolution of microcystin production is observed from the beginning to the end of the blooms (from 1.8 to 36 µg eqMcyst-LR.L-1 near the surface in 1998 for example). A potential relation with the variation of major and trace element concentrations in the water column is considered.

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF SPIROLIDE TOXINS

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Spirolides are biologically-active macrocyclic imines found in plankton and shellfish from the eastern coast of Nova Scotia, Canada. They appear in lipophilic DSP-type extracts of shellfish and cause rapid death (3-20 min) upon intraperitoneal injection into mice, with apparent neurotoxic symptomology. Several of these toxins have been structurally characterized and the primary producer of the spirolides has been shown to be the marine dinoflagellate, *Alexandrium ostenfeldii*. A rapid liquid chromatography-mass spectrometry (LC-MS) method based upon electrospray ionization has been established for the analysis of spirolides in plankton and shellfish samples. This method provides very high sensitivity (low picogram or ng/mL detection limit) and has allowed the detection of several new spirolides. Tandem mass spectrometry (MS-MS) provides structural information for the identification of such new compounds. Extraction methods for both shellfish and plankton have been developed. This includes a new micro-extraction procedure for rapid analysis of spirolides in pooled plankton cells individually isolated by micropipette or flow cytometry. Such techniques have allowed us to study the geographical and temporal distribution of spirolides in Nova Scotian waters, as well as the production of spirolides by an isolate of A. ostenfeldii grown in culture.

CHARACTERISTICS OF *ASTERIONELLOPSIS GLACIALIS* CASTRACANE BLOOM IN NEARSHORE WATERS OF COVELONG, BAY OF BENGAL, INDIA

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Occurrence of phytoplankton blooms in nearshore waters of bays is an interesting biological event in different seas. However, information on the bloom-forming planktonic diatoms is limited, in the seas around India. So an attempt has been made to study the physical-chemical and biological characteristics of the bloom of *Asterionellopsis glacialis* in the nearshore waters of Covelong, Bay of Bengal. During the period of study, temperature, pH and salinity remained more orless stable whereas dissolved oxygen concentration fluctuated between 4.9 and 9.5 ml l-1. Concentrations of nutrients viz. nitrate (13.25 μM) and silicate (200 μM) were high during the peak period of bloom, but phosphate concentration was low (0.33 μM), probably due to its utilization by the bloom-former. Chlorophyll *a*, showed two – fold increase from the initial level during the peak period of bloom (i.e. from 1.09 to 2.49 mg m3) and atthe time of termination of the bloom, it was very low (0.04 mg m3).During the peak bloom period, surface waters appeared brown due to the high concentration of *A. glacialis* cells which contributed 85% to the total phytoplankton density. Along with *A. glacialis*, diatoms such as *Bellerochea malleus, Pleurosigma elongatum, Rhizosolenia styliformis* and *Thalassiothrix frauenfeldii* were present in very low densities.Bloom of *A. glacialis* off Waltair coast was attributed to local upwelling in the Bay of Bengal associated with enrichment of nutrients and lowering of surface water temperature (Subba Rao, 1969). Bloom of*A. glacialis* in the nearshore waters of Gopalpur (Bay of Bengal) was due to limited variation in salinity and availability of more amount of nutrients and the termination of the bloom was due to nutrient depletion and the grazing pressure exerted by the copepodes (Choudhury and Panigrahy, 1989). Results of the present study have indicated that the appearance and persistence of *A. glacialis* bloom were due to hydrographical stability especially of temperature, salinity and availability of nutrients. A rapid decrease in silicate concentration from the peak period of bloom (about 5-10 fold), amidst an increase in nitrate and nitrite concentrations, resulted in the termination of the bloom. Though bloom of *A. glacialis* has not caused any harmful effects presently, such diatom blooms can cause death to the fishes through physical obstruction of the gills from excess mucus formation and anoxia. Hence a through monitoring for all marine micro-algal blooms, toxic or non-toxic, is suggested for Indian waters.

MOLECULAR DIAGNOSTICS FOR *PFIESTERIA*-COMPLEX ORGANISMS IN CHESAPEAKE BAY, USA

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The heterotrophic dinoflagellate *Pfiesteria piscicida* has been blamed for several fish kills along the US mid-Atlantic coast. In addition, it has been implicated in human health effects including neurocognitive impairment and skin lesions. *Pfiesteria piscicida* and other *Pfiesteria*-like dinoflagellates are referred to as the *Pfiesteria*-complex organisms (PCOs) . Currently the only reliable method to identify and distinguish these organisms is through tedious analysis of the thecal plate structure of cysts by scanning electron microscopy. We are doing a comprehensive survey of DNA sequences for the internal transcribed spacer (ITS) region and portions of both the small (SSU) and large (LSU) subunit genes of the ribosomal DNA complex for available PCO clonal cultures to develop DNA-based diagnostics. Sequence comparisons among the PCO cultures and to other dinoflagellates and protozoans will allow species-specific and genus-specific PCR primers and DNA probes to be developed. Clonal cultures of *Pfiesteria piscicida, Cryptoperidiniopsis* spp., " Shepherd's crook", "Lucy" and another unidentified PCO were analyzed and identified by SEM and then obtained for molecular analysis. DNA was isolated from a total of 17 PCO cultures and 3 dood source cultures. DNA sequences were obtained from the food sources to assure that PCO rather than food source DNA clones were selected for analysis. SSU-ITS and LSU DNA sequences from the PCOs were aligned and subjected to phylogenetic analyses. The 3' end of the SSU gene is highly conserved while the ITS region (excluding the 5.8S portion) and portions of the LSU gene fragment demonstrate considerablr sequence variation. Less than 5% of the sites in the SSU gene were parsimony informative while almost 50% of the sites in both the ITS and LSU regions were found to be informative. To facilitate molecular screening of the PCO cultures we are examining the culture DNAs using amplified fragment length polymorphism (AFLP) analysis. AFLP profiles will be developed for the available PCO taxa as well as food sources. Following SEM identification, culture DNAs will be subjected to AFLP analysis to identify cultures that represent the range of genetic variation within each taxa. This technique will alllow us to more effevtively target a subset of available PCO clonal cultures for the more intensive sequence analysis. As taxon-specific PCR primers and probes are developed we will test them on the available cultures and begin analysis of environmental water and sediment samples.

PERKINSOZOA, A NEW PHYLUM WITHIN THE SUPERPHYLUM ALVEOLATA?

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A hitherto unknown parasite, *Parvilucifera infectans* (Norén et Moestrup 1999) was found to infect and kill dinoflagellates, including toxic species of *Dinophysis* and *Alexandrium.* A single large sporangium (70µm) isolated from an infected cell of *Protoperidinium*, was collected by micropipetting and crushed. The crude extract was used directly in an amplification reaction using eukaryote specific small subunit rRNA primers, and thereafter cloned and sequenced. The near full length (1470 bp) sequence was aligned to representative members of the Apicomplexa, Dinoflagellata (including the parasite *Amoebophrya*), Ciliata and Perkinsea. A maximum parsimony analysis showed Parvilucifera to group with the oyster pathogen *Perkinsus*, and the two forming a sister group to the Apicomplexa. However, considering the available evidence from both molecular and ultrastructural studies, we have concluded that *Parvilucifera* and *Perkinsus* are related to both dinoflagellates and apicomplexans. The taxon is probably forming an older group within the superphylum Alveolata, an idea that earlier has been postulated for *Perkinsus.* We therefore have erected a separate phylum for the perkinsids to be named *Perkinsozoa* after *Perkinsus*, the first and best-known genus of the group.

WHAT CONTROLS THE CYANOBACTERIA BLOOM IN LAKE ULEMISTE?

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This small (volume 24*106 m3) and shallow (maximal depth 5 m, average depth 2.5 m) almost hypertrophic lake (Secchi disk depth 0.5-1.75 m and chlorophyll a concentration Cchl =13-121 mg m-3) is located close to Tallinn (capital of Estonia), an airport and a highway, but it is used as the main reservoir of drinking water for this town. The catchment area covers 1728 km2 mostly agriculture land, and extracts water from three river systems by means of a complex interlinkage of major reservoirs and canals, the only outflux is through the Water Treatment Plant. At the present there is no pragmatic, economic method of controlling algal blooms in shallow lake, where the depth of the mixed layer is determined by the physical depth of the lake and light climate is strongly affected by seasonal and long-term changes in the water level and artificially regulated water influx. Using the data of optical and biological measurements performed repeatedly in Lake ‹lemiste during years 1997-1999 we investigated the underwater light climate and its connections with concentrations of the chlorophyll a and suspended matter in the water, abundance, biomass and species composition of phytoplankton, vertical profiles of temperature and dissolved oxygen in the water as well as water level and influx. The maximum attenuation corresponded very well with the algal bloom in the lake, occurring typically at the end of August. Filamentous cyanobacteria *Limnothrix redekei* made up more than 90% of the phytoplankton abundance. There was not significant difference in abundance of that cyanobacteria in different sites of lake and their vertical profiles. The water column was well mixed and homogeneous in the whole water reservoir from May to October. Chlorophyll a and pheaophytin (strong absorption close to 680 nm) and cyanophycocyanin, pigment unique to cyanobacteria, (a very typical absorption peak at 624 nm) caused characteristic features in irradiance attenuation and reflectance spectra. The water column was well lighted during the whole observation period (most of the time the euphotic depth exceeded the mean depth of the lake). It seems that due to water influx from less productive and colder reservoirs, light controls phytoplankton growth in ‹lemiste only early in spring and in fall, but the bloom is rather controlled by water using.

EFFECTS OF A POLYAUNSATURATED FATTY ACID AND A MICROCYSTIN ON THE SURVIVAL OF *DAPHNIA MAGNA*

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In aquatic environments, polyunsaturated fatty acids (PUFAs) are generally regarded as beneficial compounds, which are necessary for the growth and reproduction of aquatic organisms. Several studies have demonstrated that the lack of certain PUFAs inhibits the production of zooplankton both under laboratory conditions and in natural waters. However, a few studies have also shown that there are PUFAs and other fatty acids with toxic properties, and it has been suggested that some of these compounds can be responsible for the toxicity of bloom forming phytoplankton. It has also been suggested that cyanobacterial strains that do not produce substances toxic to vertebrates, can still cause mortality in aquatic invertebrates due to the presence of certain PUFAs. The composition and concentrations of fatty acids in natural waters vary markedly, but compared to algal compounds traditionally regarded as toxins they have a wide distribution and occur at high concentrations in most environments. As a consequence, we suggest that the possible toxic properties of PUFAs deserve attention. In the present study, we have studied the toxicity of a PUFA to *Daphnia magna*. We have also assessed possible interactive effects with a microcystin, in order to discuss possible additive or synergestic effects of PUFAs and microcystins on zooplankton during cyanobacterial occurrences. The toxicities of the PUFA and the microcystin are furthermore illustrated by comparing the effects with toxicity data of some well known environmental pollutants, the effects of which have also been assessed on our clone of *D. magna*.

ESTERIFIED OKADAIC ACID IN NEW ZEALAND STRAINS OF *PROROCENTRUM LIMA* AND OYSTERS (*CRASSOSTREA GIGAS*)

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Prorocentrum lima (CAWD33), isolated from Rangaunu Harbour, Northland, New Zealand, produced 6.3 pg okadaic acid (OA) per cell as determined by HPLC (derivitisation with ADAM). Mouse bioassays indicated toxicity in the range of 0.4 – 0.8 MU per 106 cells. However the mouse response differed from the expected response to OA, and included neurological symptoms. Analysis by DSP ELISA test kit indicated toxicity x25 greater than was detected by mouse bioassay. HPLC analysis of strains of *P. lima* from different geographic regions in New Zealand was carried out with and without an alkaline hydrolysis step. Results indicated that OA and esterified OA were present in strains from Whatawhiwhi and Rangaunu Harbours, but not in strains from Rangiputa, in Northland. No DTX series toxins were detected, whereas a Spanish strain used as a comparison produced OA and DTX1, but no esterified OA. There was good correlation between HPLC results and those obtained by protein phosphatase (PP-2A) inhibition assay. The New Zealand strains were similar under scanning electron microscopic investigation, but differed slightly from the Spanish strain. More strains are being investigated.

INVESTIGATIONS INTO THE TOXICOLOGY AND PHARMACOLOGY OF SPIROLIDES – A NOVEL GROUP OF PUTATIVE BIOTOXINS

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In 1991, routine biotoxin monitoring of bivalve molluscs at aquaculture sites along the eastern shore of Nova Scotia, Canada revealed a novel and highly potent toxic response in mice after intra-peritoneal injections of lipophilic extracts. The symptoms in mice include piloerection, abdominal muscle spasms, hyperextension of the back, and arching of the tail to the point of touching the nose. Rapid death was observed within 3-20 minutes, preceded by neurological symptoms, including convulsions. The symptoms were very different from those associated with known shellfish toxins, including those responsible for DSP or PSP intoxication. Isolation and purification of lipophilic compounds from shellfish digestive glands revealed a novel macrocycle, consisting of a spiro-linked tricyclic ether ring system and an unusual seven-membered spiro-linked cyclic iminium moiety – hence the name spirolide. The biological origin of spirolides was later shown to be from the gonyaulacoid dinoflagellate, *Alexandrium ostenfeldii*. Assessment of the oral and intraperitoneal effects of spirolides in mice revealed an estimated toxicity of 1 mg/kg and 40 g/kg, respectively. To characterize the pharmacological effects of spirolides, mice were subjected to various drugs (e.g., atropine, physostigmine, propanolol and epinephrine), followed by a challenge with spirolide-rich *A. ostenfeldii* cell extract of unialgal cultures or purified spirolides. Some therapeutants were capable of enhancing survivability whereas others produced faster death times. After administration of *antidotes* to these therapeutants, and the reversal or enhancement of spirolide effects, at least one mode of action was identified. Spirolides affect specific receptors in mammalian systems and, at high levels in shellfish, may warrant concern for human consumers of shellfish.

FLORIDA BAY MICROALGAL BLOOMS: COMPETITIVE ADVANTAGES OF DOMINANT MICROALGAL SPECIES

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Florida Bay is a unique tropical shallow water habitat in southeastern United States that has been historically characterized by large regions of lush seagrass meadows and a clear water column supporting a low phytoplankton biomass. Significant changes in the ecosystem have been documented since 1987 that include widespread seagrass, mangrove and sponge mortalities as well as hypersaline events, and increased water column turbidity resulting from sediment resuspension and microalgal blooms. The abundance and persistence of the blooms often dominated by the cyanophyte *Synechococcus elongatus* has raised concerns about the ecological health of the bay. To examine the factors that may convey a competitive advantage to the dominant species, the light, salinity and nutrient requirements of four numerically dominant microalgal taxa of Florida Bay were examined. The effect of salinity (0-50ppt) on the growth rates of fully acclimated populations found distinct optima for the two diatom species while both blue-greens did show optima, their growth rates were largely unaffected by salinity. The acclimated growth rate in response to irradiance found the saturation irradiance of both diatom species to be approximately twice that of the two blue-green species. Phytoplankton resource-based competition between the species was examined. The equilibrium resource competition model predicted that *S. elongatus* would be the superior competitor under P-limitation. Competition experiments verified the model's predictions at salinities (S) 15,25 and 50. Competition experiments under N-limitation revealed that a diatom dominated at S=25 while the two blue-greens codominated at the low and high salinities. Minimum cell quotas of phosphorus normalized to cell volume were comparable between all four species at S=25, suggesting that the increased competitive ability of S. elongatus was not the result of a more efficient use of phosphorus.

ZOOPLANKTON RESPONSES TO PHYTOPLANKTON VARIABILITY IN A NUTRIENT ENRICHED AUSTRALIAN ESTUARY. A SIZE BASED APPROACH

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Berowra Estuary is a eutrophic drowned river valley in Sydney, Australia. The mid region of this estuary has persistently high levels of phytoplankton biomass (>15 – 60 mg.l-1) throughout the austral summer. Tidal flushing, primary production, phytoplankton and zooplankton communities were investigated to establish the processes leading to the blooms. Flushing times varied along the estuary, but were highest (7 days) at the bloom site. Primary production was highest at this site, where flushing times were sufficiently long for nutrients to be taken up and for primary production to occur. Phytoplankton taxa, over the study period were dominated by various species of *Chaetoceros, Pseudonitzschia*, *Thalasiosira* and Skeletonema. A size based approach, using image analysis technology, was used to investigate zooplankton. Zooplankton biomass (wet weight of taxa between 90-900 μm equivalent spherical diameter) in the bloom area (200 mg.m-3) was significantly greater than upstream (140 mg.m-3) and downstream (120 mg.m-3) sites. Numbers of individuals in the smallest zooplankton size classes were closely related to phytoplankton biomass. The relationship decreased with increases in the particle size classes, showing an uncoupling between phytoplankton biomass and numbers of larger zooplankton. This uncoupling was probably due to the time needed for somatic growth to occur in the zooplankton. Flushing times in the bloom reach were sufficiently long for secondary production (somatic and egg production) to take place in the zooplankton taxa present during the study. Environmental conditions (nutrients, light intensity and temperature) were such that zooplankton grazing and estuarine flushing were unable to reduce phytoplankton concentrations to below 10 μgl-1.

HPLC PIGMENT COMPOSITION OF PHYTOPLANKTON POPULATIONS DURING THE DEVELOPMENT OF *PSEUDO-NITZSCHIA SPP.* PROLIFERATIONS.

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Seawater samples were weekly collected from the Ría of Pontevedra (NW Spain) during 1998 as part of a harmful algae monitoring programme. Phytoplankton cells were identified and enumerated by light microscopy (integrated samples from 0-15 m depth). Pigment composition was determined by HPLC after size-fractionating (GFD and GFF filters). Two major proliferation episodes of *Pseudo-nitzschia* spp. were detected in March 1998 (400.000 cells L-1), and July 1998 (800.000 cells L-1). During the first maximum, *Pseudo-nitzschia* spp (50% of total diatom numbers), was mainly dominated by the toxic P. australis. Pigment analysis showed a chlorophyll (Chl) c pattern with Chl c2 as major compound, while Chl c1 and Chl c3 were minor components. Along summer proliferation, *Pseudo-nitzschia* spp (90% of total diatom numbers), was dominated by the non-toxic *P. fraudulenta.* Pigment analysis showed Chl c2 and Chl c3 as major components of Chl c family, and low Chl c1concentrations. Although Chl c3 is usually associated with members of the classes Prymnesiophyceae, Pelagophyceae and some species from Dinophyceae, it has also been detected in several Pseudo-nitzschia species as *P. fraudulenta, P. delicatissima, P. pungens* and P. *pseudodelicatissima*, butnot in *P. multiseries* and *P. australis*, species able to synthesise domoic acid, the causative agent of amnesic shellfish poisoning (ASP). Other characteristic feature of HPLC pigment analysis was the detection of a catotenoid with lycopene-type spectrum, just after the decline of *Pseudo-nitzschia* proliferation. The carotenoid was detected in the GFF (fine fraction) and its potential ecological meaning will be discussed. The parallel increase of Chl c3 values and *Pseudo-nitzschia* cell numbers (throughout the development of a quasi mono-specific *Pseudonitzschia* spp proliferation) could be used as useful additional information, but obviously, only during the time that the decision makers are waiting for the results of the official assay (i.e. HPLC domoic acid analysis), which imply a certain time-delay while the molluscs are incorporating domoic acid into its tissues.

INVESTIGATION INTO CYANOBACTERIAL BLOOMS AT LAKE MOKOAN

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Cyanobacterial blooms have been a regular feature over summer/autumn at Lake Mokoan since the late 1980's. Blooms have been dominated by Microcystis and occasionally *Anabaena* and have necessitated closure of the lake to water supply and recreational usage on a number of occasion's. The exception to this pattern occurred over the summer/autumn period in 1998, when cyanobacteria were recorded at very low cell numbers, on few sampling occasions. This respite was short-lived and *Microcystis* bloomed again over the 1998/9 summer. A regular monitoring program has been in place at Lake Mokoan since 1992 (the MSOMP), providing an eight year data set for phytoplankton and physico-chemical parameters. This data set was augmented with available information on climatic conditions and water regime to undertake a detailed investigation of factors that were consistently linked to the development of cyanobacterial blooms.

Data were investigated for patterns that linked to changes in cyanobacterial abundance and a workshop was used to identify the key factors influencing the development of cyanobacterial blooms. Important gaps in knowledge of bloom development at Lake Mokoan were also identified. The potential to manage these factors to control future blooms is discussed.

DISTRIBUTION OF PFIESTERIA PISCICIDA AND TWO ASSOCIATED DINOFLAGELLALTES ALONG THE US EAST COAST DURING THE ACTIVE SEASON IN 1998 AND 1999.

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We used PCR probes and fluorescent in situ hybridization to test for the presence of *Pfiesteria piscicida* in US east coast estuarine systems during June – November 1998 and 1999. We also probed for two associated dinoflagellate species using PCR. *P. piscicida* was widespread, and was found in every state from New York to Florida, except New Jersey. *P. piscicida* was most common in North Carolina and Maryland, two states that have experienced the most fish kills associated with *P. piscicida*. The other species were were less common in our samples. We believe that *P. piscicida* is a common inhabitant of these estuarine systems, and normally benign. Analysis of distribution patterns will include discussion of physical and chemical parameters and how they correlate to abundance.

THE HARMFUL ALGAE *CHATTONELLA ANTIQUA, C. MARINA* AND *C. OVATA* (RAPHIDOPHYCEAE) ARE PHYLOGENETICALLY THE SAME SPECIES

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Chattonella antiqua (Hada) Ono, *C. marina* (Subrahmanyan) Hara et Chihara and *C. ovata* Hara et chihara, Raphidophyceae, are typical red tide flagellates in Japan and are known to be fish-killing microalgae. In recent years, these red tide incidents have increased in geographical distribution, such as in Australia and some European countries. It is, therefore, very important to identify these species precisely. However, the morphological taxonomy of these species are not easy because their whole shapes can be easily variable and changeable, even with clonal culture. For phylogenetic analysis and molecular identification of the noxious red tide forming flagellates *C. antiqua, C. marina, C. ovata, C. verrculosa* and *Heterosigma akashiwo*, we analyzed 18S and 28S rRNA gene sequences of these species. Each five isolates of *C. antiqua* and *C. marina* and two isolates of *C. ovata* from geographically different costal waters of Japan had exactly the same sequences of 18S rDNA. Moreover, 28S rDNA D1/D2 domain (728bp), which are less conservative, were also completely identical, revealing the genetic homogeneity and the same species. Phylogenetic analysis based on 18S rDNA sequences indicated that *C. antiqua, C. marina* and *C. ovata*, which are phylogenetically the same species, were closely related to *H. akashiwo* than to *C. verrculosa*. This result was supported by the phylogenetic analysis using 28S rDNA D1/D2 regions. The phylogenetic study of Rhaphidophyceae stresses the need for the reconsideration of morphological taxonomy of genus *Chattonella.*

FLUORESCENT IN SITU HYBRIDIZATION WITH RRNA-TARGETED PROBES FOR *ALEXANDRIUM TAMARENSE* AND *A.CATENELLA* IN NATURAL POPULATION

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The toxic dinoflagellates *Alexandrium* spp. causing paralytic shellfish poisoning (PSP) are identified on the basis of morphological features. Recently, however, it has been pointed out that these features often vary in response to the changes of environmental conditions and these morphological criteria don't reflect in phylogenetic relationship. In this study, we established easy and objective method of identification for cultured *Alexandrium* spp. by using Fluorescent in situ Hybridization (FISH) with rRNA-targeted fluorescent probes, and furthermore tried to improve this method for natural population. Fluorescent oligonucleotide probes targeted against D2 region of the 28S rRNAs of *A. tamarense* and *A. catenella* were prepared and applied to cultured *Alexandrium* spp. and many other algae. Each probe for both species were highly specific for particular strains of *A. tamarense* and *A. catenella* and these individual cells could be detected within only one hour. Moreover in flow cytometric analysis, quantification of these FITC-labeled cells was possible. The specific detection of these cells in natural population by FISH method, however, was very difficult by this method because of some autofluorescence, aggregation and disruption of *Alexandrium* spp. cells and the other organisms. FISH method was improved for the field samples. First, the decolorization with acetone was added to the protocol. Second, CTAB treatment was abolished. Third centrifugations on the all processes were replaced by filtration. As a result, more than 75% cells of *A. tamarense* and *A. catenella* could be detected within one hour.

GENETIC AFFINITIES OF THE AUSTRALIAN PSP DINOFLAGELLATES *ALEXANDRIUM CATENELLA, A. TAMARENSE* AND *A. MINUTUM*: INTRODUCED OR ENDEMIC?

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The genetic relationships of five strains of *Alexandrium catenella*, three of *A. tamarense* and five of *A. minutum*, representative of Australian and New Zealand populations of these dinoflagellates, were examined using a variety of methods. Analysis of both the D1-D2 region of the large-subunit (LSU) ribosomal RNA gene proved an adequate technique for the study of *A. catenella* and *A. tamarense*, but not *A. minutum*. However, the use of the 5.8S small-subunit (SSU) gene with its flanking internal transcribed spacer (ITS) regions 1 and 2 proved adequate for all strains studied. Sequence analysis of fragments of the SSU and LSU rDNA indicate that Australian and New Zealand populations of *A. catenella* are genetically homogeneous. However, *A. tamarense* exhibited three distinct genotypes, and *A. minutum* exhibited a further two. Analysis of the toxin composition of a number of strains within Australia, in comparison with published toxin profiles support these results. It is thus hypothesised that Australian and New Zealand populations of *A. catenella* may have been introduced from temperate eastern Asia (Japan) in recent times.In contrast, the genetically unique Tasmanian strain of *A. tamarense,* and Eastern Australian and New Zealand strains of *A. minutum* most likely reflect indigenous toxic dinoflagellate populations.

A PHOTODYNAMIC SENSITIZATION INTERFERING IN THE MOUSE BIOASSAY FOR DSP

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Quality surveillance of the waters in the production areas of bivalve mollusc in Galicia (NW Spain) is performed by our unit, the CCCMM (Marine Environmental Quality Monitoring Center). This surveillance includes monitoring of oceanographic conditions, biotoxins and phytoplankton. In the course of routine DSP monitoring, using the mouse bioassay of Yasumoto 1978, we have observed strange symptoms in some mice injected with DSP extract, notably photosensitivity and marked fluid discharge between the skull and the skin. Deaths occurs between 18 and 48 hours after injection. Histopathological analysis reveals marked congestion in all organs, and severe damage to the liver and spleen, all consistent with the action of a toxic compound. The presence of PSP, OA and derivatives, PTX, YTX, ASP and KT-3 was ruled out. Simultaneous routine phytoplankton counts did not reveal the presence of any harmful species known associated with these observations. The putative toxic responsible for the observed symptoms was soluble in acetone, in ether and in 80% MeOH, but not in hexane.

Further studies revealed that the symptoms can be prevented if, after injection, the mice are kept in the dark. This observation is consistent with the presence in the samples of a phototoxic, leading us to suspect a pyropheophorbide like compound. Chromatographic determination confirmed the presence of pyropheophorbide a at a concentration in excess of 0.5 mg/g hepatopancreas. Intraperitoneal injection of pyropheophorbide a into healthy mice provoked the same strange symptoms as observed in the DSP bioassay, strongly suggesting that these symptoms are at least partially due to this compound.

HUMAN TOXICATION BY MARINE BIOTOXINS IN PORTUGAL, TWO CONFIRMED CASES

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Having bivalves as vectors we have been informed that in 1994 several cases of PSP syndrome had occurred at Ericeira , and that the DSP syndrome was detected in Loulé in 1998. The PSP toxication occurred on the 18 of October 1994 after eating blue mussels (*Mytilus edulis*) picked up at Ericeira beach, several people went to the regional hospital and nine of them were so badly ill, with neurological symptoms, that they had to be transported to Santa Maria Hospital in Lisbon. For the DSP syndrome, wich occurred on the 12 of February 1998, the responsible bivalves were donax clams (*Donax trunculus*) harvested at Fuzeta/Olhão (Algarve litoral) and eaten by nineteen Loulé Health Care Unit employees and relatives. All the affected people got abdominal cramps, headaches, vomits and diarrhoea after 6 hours of ingesting the donax clams. In both cases IPIMAR as the National Reference Laboratory for Marine Biotoxins, was contacted and samples from the left over of the respective bivalves were asked for analysis. Data on toxicity, responsible phytoplankton species and toxications are presented.

CHARACTERIZATION OF THE CONJUGATES OF PARALYTIC SHELLFISH TOXINS AND THIOLS FORMED IN THE COURSE OF REDUCTIVE TRANSFORMATION OF GONYAUTOXINS TO SAXITOXIN

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In the course of the transformation of gonyautoxins (GTXs) to saxitoxins (STXs) by the treatment of thiols such as glutathione and 2-mercaptoethanol, most of GTXs disappeared from the reaction mixtures before the formation of STXs, indicating that the intermediate is formed in the reaction. The compound newly appeared in the reaction mixture was purified by repeated column chromatography on Bio-Gel P-2. Further reduction of the purified compound with excess amounts of thiols resulted in the formation of STXs, showing that the compound is the intermediate. Spectral analysis showed that the compounds were the conjugates of STXs and thiols in which these two molecules are bound by covalent linkage between C11 atom of STXs and sulfur atom of thiols.

DEVELOPMENT OF NOVEL INSTRUMENTATION FOR AUTONOMOUS COLLECTION AND REAL-TIME DETECTION OF HARMFUL ALGAE

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Development and application of species-specific molecular probes has been heralded as one means to speed and ease the detection and quantification of a wide range of harmful algal bloom (HAB) species. In turn, it is assumed that our ability to view HAB events in the context of ocean physics and chemistry in near real-time will improve dramatically, as will our capacity for processing large numbers of samples rapidly. However, application of the probes for routine analysis of natural samples is presently hindered by the need for repetitive operations that typically demand trained personnel and specialized laboratory facilities. These requirements severely restrict the utilization of molecular probes for large scale ecological studies because the rate of sample processing is in many cases limited and application of the technology outside of a laboratory setting is difficult, if not impossible. In an effort to overcome these problems a novel instrument was designed to collect discrete water samples autonomously, concentrate particles contained within those samples onto filter disks, and automate application of species-specific DNA probes to identify and quantify particular organisms so captured. In addition to archiving discrete samples, the instrument is also capable of transmitting results of the probe assays in real-time to a remote location for data processing and interpretation. This presentation summarizes the development of this new tool, its use to date, and potential future applications.

GROWTH STUDIES AND LIFE STAGES OF THE DINOFLAGELLATE *CRYPTOPERIDINIOPSIS* SP VS. *PFIESTERIA PISCICIDA*

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Cryptoperidiniopsis sp. was isolated from sediment samples collected in Chesapeake Bay tributaries. It was identified by Karen Steidinger through SEM and by Parke Rublee through a genetic probe and appears to be distinct from *C. brodyii*. Its life history is complex and includes zoospores, gametes, planozygote, cysts, and amoeboid stages. It is also capable of heterotrophic feeding. This *Cryptoperidiniopsis* sp. (DEQ-002) and a *P. piscicida* strain provided by JoAnn Burkholder were each placed into three 250mL culture vessels containing f/2-Si medium with *Cryptomonas* sp. (CCMP #767 Provasoli-Guillard) as a food source. The inoculum of dinoflagellates was adjusted so that the initial concentration was always 500 mL-1. Samples and controls were run in triplicate. Factors investigated include temperature, salinity, food concentration, and fish toxicity. Toxicity to fish (tilapia) was tested in a biohazard level 3 facility by placing 25 mL of dinoflagellates into 10 L culture vessels containing fish. In its logarithmic growth phase the dinoflagellate can double every 8-10 hours. The growth rate of *Cryptoperidiniopsis* sp. was found to be high (F = 1.43) when compared to other dinoflagellates, including *Pfiesteria piscicida* (F = 0.84). Both dinoflagellates can feed with a peduncle on a wide variety of algae, but *Cryptomonas* was preferred as a food source by each. In both species the maximum dinoflagellate abundance was related to increased concentration of algal prey, as well as mesohaline salinities and warm water temperature (>15C). This *P. piscicida* strain was found to be positive for fish toxicity. The *Cryptoperidiniopsis* sp. from Chesapeake Bay tributaries was negative for fish toxicity having killed no fish over a 10 week period. Supported by the Virginia Dept. of Health, and Virginia Dept. of Environmental Quality.

THE PRODUCTION OF YESSOTOXIN BY *PROTOCERATIUM RETICULATUM.*

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Yessotoxin has been responsible for several shellfish toxicity events within New Zealand. The dinoflagellate *Protoceratium reticulatum* is thought to be responsible for the occurrence of this yessotoxin. We aim to establish at what stages of a *P. reticulatum* bloom yessotoxin toxicity is the greatest and to find out which derivatives of yessotoxin are produced by *P. reticulatum* at different stages of growth and under different environmental conditions. This information is important for effective phytoplankton monitoring programs. We have established standard culture conditions and measured cell growth under varying environmental conditions for *P. reticulatum.* The most effective growth medium was GP, a seawater medium containing soil extract. The combination of light intensity and temperature that produced the most rapid growth was 45μmolm-2s-1 and 21 C. The lag, exponential and stationary phases of growth have been defined. Culture samples of *P. reticulatum* are now being analysed by HPLC to quantify the amounts of yessotoxin and its derivatives produced at different growth stages and in various environmental conditions. The results of this analysis will be presented.

THE DEVELOPMENT OF HEPATOTOXIC CYANOBACTERIAL SCUMS AND BLOOMS

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The universal view of microcystins as hepatotoxins does not take into account the fact that cyanobacteria have existed for up to 3.5 billion years, long before the appearance of higher organisms. The assumption that cyanobacteria in their early stage of evolution already produced microcystins is, of course speculative but on the other hand, it is unlikely that they developed these complex substances just as defence mechanism against potential predators. The overall logic of evolution is principally based on egoism rather than agressivness, and we can therefore expect organisms to be stimulated to produce substances that enable better adaptation rather harm rivals.

Microcystins amplify the cyanobacterial bloom formation in different but possibly closely related pathways. They enhance cell growth by means of a higher proliferation rate and by increasing their volume. Higher division rates are enabled by the presence of a larger photosynthetic apparatus. Larger cells with a larger photosynthetic apparatus, like the toxin producing cells, are more competitive at increased levels of turbidity, as is the case in cyanobacterial blooms. Therefore producing colonies are able to prevail in a bloom that can start from non-producing strains of cyanobacteria. Planktonic algae are able to adapt to low light intensities by increasing the sizes or the numbers of their photosynthetic units. Our results support the theory, that in cyanobacteria microcystin production is connected to this regulatory mechanism. The microcystin content of a single cell can exceed the amount of the chlorophyll a that is of paramount importance for growth and proliferation of autotrophic organisms. This fact points to an involvement of microcystins in primary cellular processes.

Sedmak, B. and Kosi, G. (1998) The role of microcystins in heavy cyanobacterial bloom formation. J. Plankton Res., 20, 691-708.

ROTATIONAL DIFFUSION OF SWIMMING MICRO-ORGANISMS IN A VISCOUS FLOW

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A new method is presented for calculating the rotational diffusivity of small particles suspended in an ambient flow and subject to both external torques and rotational Brownian motion. The problem is motivated by the study of dilute suspensions of swimming micro-organisms that generate macroscopic patterns known as bioconvection. The individual micro-organisms are assumed to swim at a constant speed (although there is variation across the population) and their orientations are determined by a balance between viscous torques due to the ambient flow and gravitational torques due to offsets in their centres of mass relative to their centres of buoyancy. This effect, which is known as gyrotaxis, is modified by the rotational Brownian diffusion.

The numerical values of diffusion coefficients for a fluid containing swimming micro-organisms are explicitly needed in continuum models of bioconvection. However, they have never been calculated and only ad hoc approximations are used, presumably because of the apparently lengthy computations required. In this paper, a novel approach yields an explicit expression for the diffusivity tensor. A reasonable agreement with experimental data obtained for the swimming alga is found.

IN VITRO FORMATION OF CHROMISTA ALGAL BLOOMS BY TREATMENT WITH ANTIBIOTICS.

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The connection between certain algal blooms and natural and man-made chemicals including antibiotics has been a subject of considerable interest, but no concrete data are seen in literature. We carried out a simple experiment which yielded some striking results. The local, Narragansett Bay, seawater and sea sand collected at the same location in January were placed in Fernback flasks and spiked with F/2 nutrients. Three sets of flasks, first challenged with ampicillin (20 μg/ml, twice), second with oleandomycin (20 μg/ml, twice) and a control without antibiotics, were kept in an incubator at 20 degrees C under illumination and observed over a long period. The flora in the control flask followed successions of different diatom blooms and ended up mostly with a steady mixture of filamentous blue-green algae. The ampicillin-treated flask had a diatom blooms initially, followed by two different Cryptomonad blooms (~3rd week), a dense Rhaphidophyceae (*Olisthodiscus)* algal bloom (~10th week) and a dense Haptophyta *(Pleurochrysis*) algal bloom. The oleandomycin-treated flask remained almost in a sterile condition until the 10th week, when a round yellow flagellate (*Phaeocystis*) appeared and it developed in mats of dense colonies. After 30 weeks, some Coccolithophorids appeared. Ampicillin is a beta-lactam antibiotic, which inhibits the bacterial cell wall biosynthesis, may have changed the ecology in the flask by killing bacteria including the prokaryotic blue-greens, thus giving chance for the Chromista organisms to grow. Oleandomycin is a macrolide antibiotic which inhibits protein biosynthesis by acting on the bacterial 50s ribosomes. Our experimental results clearly shows that it also affects algae including diatoms suggesting that they also may have drug targets similar to the 50s ribosome. This finding may have some important implications, but, in our laboratory, it is currently applied to make Chromista algal isolates. It is an easy and effective way to find algae in this category other wise hard to find. Supported by NIH grant GM28754 and CA67775.

MARINE BIRDS AND HARMFUL ALGAL BLOOMS: SPORADIC VICTIMS OR UNDER-REPORTED EVENTS?

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"Seabird wrecks"--any much larger than usual concentration of seabird corpses washed ashore over a short period of time--often provide evidence of deleterious conditions in offshore populations, e.g. weather, food, pollution, fishing activities, and parasites. It is noted in the literature that wrecks caused by natural toxins such as botulism and algal toxins are apparently less common; however, this perception may be due to a combination of factors including the bird species involved, size of populations, location, and chance of discovery. Wrecks involving near-shore species probably provide a more accurate estimate of total mortality for any given event. Mortalities of marine birds in association with blooms of toxic algae have been reported from geological times to the present. Given the long historical presence of harmful algal blooms worldwide and the numbers of seabirds that feed on filter-feeding fish and shellfish, it is surprising that so relatively few incidents of sea bird death as a result of toxic algae have been reported. Often these reports are anecdotal but tend to be more complete for scarce species and rare events. Factors working in concert can also lead to wrecks that might not occur as a result of any of the factors working independently, e.g. starvation tends to render birds more vulnerable to stress. A survey of available data on the impacts of toxic algae on seabirds has revealed an array of responses ranging from reduced feeding activity and loss of motor coordination to death. Severe impacts on recruitment have been noted in some populations. There are few experimental studies; however, evidence has been provided for the ability of some species to ëlearní to avoid toxic food sources. A summary of available data on seabird/toxic algal interactions is presented and suggestions for observation and recording practices with respect to impacts on seabirds during future blooms of harmful algae are offered.

STATUS OF HARMFUL ALGAL BLOOMS IN INDONESIAN WATERS

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The occurrences of harmful algal blooms in Indonesian waters have been increasing since the beginning of the first red tide programme in this country in 1991. Some of the blooms have caused illness and even death of people and also environmental deteriorations and economic losses in fisheries. The number of red tide species and distribution of those species also increased coincident with the raising number of investigations and trained people which existed in every province of this country.
Some species found have been observed as red tide maker and some of those are known as toxic species. Most of toxic species found are belonging to the dinoflagellates, however, only a few have caused bloom phenomena such as *Pyrodinium bahamense var. compressum* and *Alexandrium affine*. The most frequent species which have caused red tide phenomena in certain areas were belonging to the cyanobacteria, mainly *Trichodesmium erythraeum*, dinoflagellate *Noctiluca scintillans* and diatom *Chaetoceros spp*. The outbreaks of those species appeared occasionally in certain areas. The list of HAB species that have been recorded in Indonesian waters since the beginning of the programme is as follows: *Pyrodinium bahamense var. compressum, Gymnodinium catenatum, Alexandrium affine, A. minutum, Gonyaulax spinifera, Dinophysis caudata, D. rotundata, Noctiluca scintillans, Prorocentrum lima, Gambierdiscus toxicus, Pseudo-nitzschia pungens, Chaetoceros convolutum,C. concavicorne, Trichodesmium erythraeum and T. thiebautii.* The occurrence and distributions of those red tide species in Indonesian waters will be discussed.

THE IMPLICATIONS OF *ALEXANDRIUM TAMARENSE* RESTING CYSTS IN AN AREA OF SHELLFISH AQUACULTURE IN IRELAND

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The Irish Marine Institute's Fisheries Research Centre carry out a monitoring programme for the detection of algal toxins in shellfish. This programme is carried out under EU Directive 91/492. During the course of this programme the North Channel area of Cork Harbour has been the only location in Ireland where toxins causing Paralytic Shellfish Poisoning (PSP) have been detected in shellfish above the regulatory limit. For short periods during each of the summers of 1996, 1997 and 1998, PSP toxins were found in mussels (*Mytilus edulis*) from this area above the regulatory limit period necessitating a ban on harvesting. Oysters (*Crassostrea gigas*) from the same area remained below the regulatory threshold. The dinoflagellate *Alexandrium tamarense*, a known vector of PSP toxins, was observed in the area during each of the toxic events. The exact origin of the populations of *A. tamarense* was unknown. *A. tamarense* is known to produce a cyst stage as part of its life cycle. These cysts can remain viable in the sediments for several years. A survey of the distribution of cysts of *A. tamarense* in the surface sediments in Cork Harbour was carried out in order to determine if they were potentially seeding the area. They were detected in 6 sites, and sucessfully germinated to yield vegetative cells. The results of the survey are presented and discussed.

SEAFOOD TOXINS IN ZANZIBAR, EAST AFRICA

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Interviews with fishermen and traditional healers indicate that villagers on the Zanzibar Islands (Unguja and Pemba) regularly avoid consuming particular types of fish, or parts of those fishes that can sicken and sometimes kill. The western-trained medical community appears largely unaware of these avoidance patterns and the potential exposure of tourists, who may be more at risk than locals. This presentation will describe results from the interviews indicating the types of poisonous fish and the symptoms that result from their consumption. The dangers appear largely to be those expected from ciguatera toxins. The suite of phytoplankton responsible are poorly known, with the exception of Prorocentrum lima, *Gambierdiscus* sp., and *Pseudo-nitzschia* sp. Although local fishers and villagers are hesitant to acknowledge problems with seafoods, none-the-less consumption patterns appear largely to protect local populations from illnesses stemming from harmful algae. These avoidance patterns and the existence of more common and life-threatening medical problems have reduced the visibility of dangers from toxins in seafoods. In Zanzibar, and possibly in other coastal communities in the developing world, these cultural practices and other health issues likely account for serious under-reporting of harmful algal events, a topic that will be briefly discussed in this presentation.

CYANOBACTERIAL HEPATOTOXINS DO NOT ACCUMULATE IN BALTIC SALMON OR HERRING?

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Nodularin (NODLN) is a cyclic pentapeptide hepatotoxin that is closely related to microcystins (MCs). This toxin is produced by a toxic brackish water cyanobacterium *Nodularia spumigena* which regularly forms blooms in the Baltic Sea. Herring (*Clupea harengus*) were caught every 3-4 weeks in the northern Baltic Sea between July and September 1997. Salmon (Salmo salar) were caught in the same area between March and October 1997 and from the Gulf of Riga in August 1997. The total toxin content in herring muscle and salmon liver was measured with an ELISA assay. Although there were exceptionally heavy blooms of toxic *Nodularia* in the Baltic Sea in summer 1997, results showed that the samples contained only very low quantities of NODLN or MC. The concentrations were a magnitude lower than those we found in the livers of Baltic flounder and cod in 1997. It seems that only insignificant amounts of NODLN or MCs accumulate in Baltic herring and salmon.

AMINO ACID PROFILES IN SPECIES AND STRAINS OF *PSEUDO-NITZSCHIA* FROM MONTEREY BAY, CA: INSIGHTS INTO THE METABOLIC ROLE(S) OF DOMOIC ACID

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The phenomenology of domoic acid (DA) production by strains of the *Pseudo-nitzschia* species complex has received considerable attention, leading to general observations that DA accumulation is stimulated by growth limiting or stress conditions. Although DA is a structural analogue of proline, no direct evidence is available linking DA and proline metabolism in *Pseudo-nitzschia*. In order to ascertain whether DA behaves as a functional analogue of proline, the free amino acid (FAA) composition of 5 species and 20 strains of *Pseudo-nitzschia* spp. from Monterey Bay were obtained by HPLC-UV profiling of their phenylthiocarbamyl amino acid (PTC-AA) derivatives. DA accumulation varied by 2-orders of magnitude among independent isolates of *P. multiseries* and *P. australis*, with the isolates of the latter species exhibiting consistently higher cellular yields of DA. Proline content was lower in cells accumulating high levels of DA (>1 fmole/cell) indicating a regulatory switch occurs between the biosynthetic pathways for these amino acids. All *Pseudo-nitzschia* species accumulated large pools of taurine (50% of total FAAs) when grown in Monterey Bay seawater (34 ppt). This osmolyte was not detected in other diatom species when grown under equivalent conditions. These trends indicate that the genus Pseudo-nitzschia may be characterized by a hypersensitive phenotype with respect to oceanic salinities. As taurine content covaried with DA accumulation in *Pseudo-nitzschia,* it may provide a useful biomarker for potentially toxic bloom events. Interactions between DA, proline and taurine metabolism will be discussed.

EVIDENCE FOR CYANOBACTERIAL TOXICITY AT AUSTRALIAN PRAWN FARMS

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Cyanobacteria can often dominate phytoplankton blooms at marine prawn farms and it is generally believed that this is not detrimental to the health of prawns. In fact, traditional culture of marine prawns in Asian countries is reported to have depended on the growth of benthic cyanobacteria (lab-lab). However, there have been reports of associations between mass mortalities of cultured prawns and blooms of cyanobacteria from species of Oscillatoriales. This paper reports on the results of bioassays in which black tiger prawns, *Penaeus monodon*, were given intramuscular injections of filtered extracts from a cyanobacterial bloom. One of the main organs of interest were the hepatopancreas; a complex organ located in the cephalothorax and is equivalent to the liver of vertebrates. At sublethal doses, the hepatopancreas appeared abnormal – with light and dark areas, a lumpy texture and was sometimes ruptured. Histological examination revealed that cells of the hepatopancreatic tubules were in varying stages of breaking away from the basement membrane and sloughing off into the lumen. The observations suggest that an hepatotoxin was present in the cyanobacterial blooms The other organ of interest was the compound eye ˆ a complicated structure that is responsible for vision and endocrine secretions. Edema and malacia were observed in the ganglion of the eye. It is concluded that a neurotoxin was present and this could contribute to mortality in cultured marine prawns.

THE ADVECTION OF A TOXIC BLOOM OF *GYMNODINIUM CATENATUM* TO THE GALICIAN RIAS, DETECTED FROM SATELLITE IMAGES

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Coastal conditions in Galicia at the end of the 1995 upwelling season are presented. Winds, sample data from the monitoring in the Galician Rias and SST images from NOAA satellites are combined to study the coastal patterns when a toxic algae bloom of *G. catenatum*, produced PSP toxicity levels above legal limits in cultured shellfish.

After 20 days of northerly winds, the wind direction changed to be from south, increasing its intensity. CTD data showed a generalised downwelling in the Rias which produced, in the outer station of Ria de Vigo, the sinking of the 17C isotherm and the maximum of fluorescence until almost 40m depth. *G. catenatum* was found in low concentrations in the inner part of the Rias, the maximum concentration in the external part of the Ria de Vigo (the southernmost one), with more than 30000 cells/l coincident with the strong downwelling. *G. catenatum* was not present in previous samples, which supports the idea of its advection in the shelf water that was transported into the Rias. The temperatures measured in the Rias are similar to the offshore water temperature in the previous upwelling period, suggesting that offshore water is moved onto the coast, but SST images show a different pattern in the area. Warm water showing a northward movement was found at 15-25 km by the coast, in conformity with the measured southerly winds and the CTD data. Nevertheless, upwelling features were yet presented over the shelf break, indicating an equatorward movement of cold water. This equatorward flow blocked the entrance of the oceanic offshore water into the Rias. In such situation, water that was moved onto the rias with southerly winds was not offshore water of the same latitude, but south water that was transported northward following the coast from Portuguese latitudes.

EVOLUTION OF A *GYMNODINIUM BREVE* RED TIDE BLOOM ON THE WEST FLORIDA SHELF: RELATIONSHIPS WITH PHYSICAL FACTORS

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The Ecohab:Florida program conducts monthly quasi-synoptic cruises within an area on the West Florida Shelf that extends from Tampa Bay to Ft. Myers. Standard hydrographic measurements are made at 63 locations along three cross-shelf, one diagonal, and one along isobath transects. A bloom of *G. breve* was first detected off Ft. Myers in November 1998 and persisted through January 1999 in this area. In February 1999, cell counts indicated that the bloom had moved north to the Tampa Bay/Sarasota region and the populations off Charlotte Harbor had disappeared. Examination of the temperature, salinity, and sigma-t data for transects taken before and after November suggest that the bloom developed after the loss of a strong thermocline and the water column became isothermal and isohaline. This pattern persisted in coastal waters including the Tampa Bay/Sarasota region through February. Continuous underway T, S, and fluorescence, moored current meter and meteorological records were examined to determine if the bloom was transported northward or had arisen off Tampa Bay de novo. The presence of a thermal front along the 15-20m isobath formed a seaward barrier that confined the bloom to nearshore waters along the 10m isobath. Current meter data suggests that northerly flow along the 10m isobath over a three-week period was sufficient to transport the bloom 120km from Charlotte Harbor to Tampa Bay.

DISTRIBUTION OF POTENTIALLY TOXIC CYANOBACTERIA AND BACTERIA IN THE COURSE OF ARTIFICIAL RECHARGE OF GROUNDWATER

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The occurrence of cyanobacterial blooming is typical for the Lake Mazais Baltezers, which is the water source for artificial groundwater recharge plant supplying to 25% of drinking water for Riga City. The investigations of algae and bacteria were carried out in 1997-98 in the lake as well as in water, sediment and vadose zone of infiltration basin. The blooms of Cyanobacteria appear in water of the lake and infiltration pond in July (30400 and 2768400 cells l^{-1} or 0.381 and 0.847 mg l^{-1} correspondingly) when *Microcystis* spp. is dominating, and in August (888200 and 17997300 cells l^{-1} or 0.847 and 1.925 mg l^{-1} correspondingly) when the mass blooming of *Aphanizomenon flos-aquae* is stated. The development of algae as well as bacteria (psychrophillous, mesophillous and oligocarbophillous aerobic and facultatively anaerobic saprophytes, total bacterial number) in water of infiltration basin is substantially higher. Cells of Cyanobacteria are observed to the depth of 3cm under the contact layer ìwater-sedimentî in infiltration basin, and in contrast to water, Cyanobacteria in sediment are detected not only in the late summer, but also in other seasons. The credible correlations between the cell numbers of Cyanobacteria and saprophytic bacteria of different groups, and total bacterial number are not established in water as well as in sediment. The investigations of 10 bore-holes (on the way from infiltration pond to siphon-pipe) with the depth to 900cm has shown that Cyanobacteria develop only in the upper layer of infiltration pond while bacteria are stated up to the depth of 900cm in all bore-holes.

INTERACTIONS BETWEEN TWO COMMERCIALLY IMPORTANT SPECIES OF BIVALVES AND THE TOXIC ESTUARINE DINOFLAGELLATE, *PFIESTERIA PISCICIDA.*

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The toxic, estuarine dinoflagellate, *Pfiesteria piscicida,* has been implicated as a causative agent of major fish kills along the eastern coast of the U.S.A. *Pfiesteria* zoospores are unique in that they exhibit directed attack behavior towards live finfish and produce toxin(s) which strip epidermal tissue from finfish and impair the nervous system, leading to paralysis and suffocation. Here we present the first report of directed attack behavior by *P. piscicida* zoospores toward larval pediveligers of the bay scallop, *Argopecten irradians*. A weaker attack response toward pediveligers of the eastern oyster, *Crassostrea virginica,* was also observed. Within 5 minutes of zoospore introduction into culture with larval pediveligers (21 oC, 15 psu, zoospores were observed to congregate around the individual larvae and attach via their peduncles. Within 15 minutes, the zoospores had penetrated into the visceral cavity of the shellfish larvae and had begun to feed aggressively upon exposed tissue. After 30 minutes, all shellfish tissue except the adductor muscle had been consumed, leaving a hollow cavity in which the zoospores encysted. Only the pediveligers which had discarded their velums were preyed upon by the zoospores. Those larvae with active, extended velums appeared to discourage the zoospores, attack and feeding behavior. In contrast to these observations with larvae, adult shellfish were observed to actively filter *Pfiesteria* zoospores out of suspension. Examination of the faeces produced by the shellfish indicated that the zoospores had formed temporary cysts and passed through the digestive tract with no apparent adverse affects on cell viability. Within three hours, 90% of the cysts excysted and regained motility. The data indicate that *Pfiesteria* zoospores have the potential to adversely affect larval recruitment and survival.

ECOHAB: FLORIDA – AN OVERVIEW

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The federal program known as Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) funded two regional programs in its first year: Gulf of Maine and Gulf of Mexico (later called ECOHAB: Florida). The goal of ECOHAB: Florida, now in its third year, is to predict the development, growth and transport of *Gymnodinium breve* on the west Florida shelf and in adjacent waters. In addition to modeling the large-scale loss and gain factors in this ecological subsystem, this multi-institutional program will predict landfall; measure *G. breve*'s behavioral and physiological responses and photoadaptations to nutrients, salinity and light; study the fate and effects of brevetoxins in the marine environment; identify life cycle and cell stages; determine the significance of prebloom conditions; establish the trophodynamics of *G. breve*; determine cell cycle regulation, growth and production; and design a cell surface recognition probe. Data have been collected for more than one year from monthly offshore cruises that occupy 59 fixed stations, monthly onshore-offshore transects that occupy seven stations and a yearly three-week process cruise during blooms. In 1999, three red tide blooms occurred simultaneously in coastal Florida waters: in the northwest, southwest and northeast. Each year of the program, a red tide has occurred off Florida. In addition to the cruises, automated buoy data (e.g., salinity, temperature and currents) is being collected to look at shelf circulation. Also, the analysis of >35 years of historical red tide data, in relation to *Trichodesmium* blooms and atmospheric iron deposition, suggests that iron deposition preceding Trichodesmium blooms on the west Florida shelf might trigger large-scale *G. breve* blooms.

A REPORT OF TOXIC MICROALGAE IN FLORIDA WATERS

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Florida has 8,000 miles of coastline,occupies 59,988 square miles of land, and has an unusually high diversity of toxic or potentially toxic marine and freshwater microalgae. It also has a high diversity of other plant/animal species in marine and freshwater habitats that vary from coral reefs to salt marshes to a unique freshwater system known as the Florida Everglades has many endemic species. The state itself crosses six degrees of latitude and goes from tropical to warm temperate. The toxic species include 27 dinoflagellates (*Gymnodinium, Gyrodinium, Gambierdiscus, Prorocentrum, Ostreopsis, Coolia, Dinophysis, Phalacroma, Alexandrium, Gonyaulax, Pfiesteria*), 6 flagellates (*Chattonella, Fibrocapsa, Heterosigma, Prymnesium, Chrysochromulina*), 3 diatoms (*Pseudo-nitzschia*), and > 15 blue-greens in 9 genera. Of the toxic marine species, 67% are dinoflagellates with about half of those being benthic species. Many of the Florida strains have been shown to be toxic by isolating, culturing and testing for specific toxins. An interesting point about the presence of known toxic species is that the harmful effects are not always realized. For example, a cell count of 7 million *Gymnodinium breve* cells/L may not kill baitfish that are in the same water, a cell count of 6 million of *Pseudo-nitzschia pseudodelicatissima*/L may not cause shellfish poisoning and, even *Chattonella* blooms that produce brevetoxins may not be associated with fish kills or toxic shellfish. Even a toxic species of *Alexandrium* may not cause PSP. In order to assess public health and environmental risks, presumed toxic species need to be isolated and tested for production of toxins or bioactive compounds such as fatty acids, and there needs to be a long-term HAB monitoring program supplemented with public health information (number of intoxications, symptoms) and aquatic health (fish and invertebrate kills or diseases)data. Florida has such a program for *G. breve* HABs and is developing a program for other HAB species.

RECREATIONAL EXPOSURE TO CYANOBACTERIA IN THREE SOUTH EAST QUEENSLAND LAKES

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Preliminary results of a three year prospective cohort study will be presented. The study aims to identify and quantify acute respiratory, dermatological and gastro-intestinal symptoms resulting from recreational exposure to cyanobacteria. Volunteers who engage in freshwater recreation and are not exposed to cyanobacteria will form the control sample. Day visitors to designated recreation sites at three South East Queensland reservoirs who plan to swim, ski or sailboard will comprise the study group. Volunteers will complete a questionnaire on study days to provide information on water exposure, recent illnesses and recent prior water exposure. Phone follow-up will be conducted after four days. Water will be sampled at various sites within recreation areas at three times through the day, to account for temporal variation in cell densities. Cyanobacterial species will be identified and counted. Water samples will also be analysed for bacterial enteric pathogens. Cercarial infestation (Swimmer's Itch), a potential dermatological confounder, will be flagged by specific questions at follow-up. The strengths and limitations of the study design will be discussed. This study is funded by a grant from the South East Queensland Water Board.

INFLUENCE OF RIVERINE DISSOLVED ORGANIC MATTER ON THE NITROGEN USE AND TOXICITY OF *PRYMNESIUM PATELLIFERUM*

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Prymnesium sp. blooms have caused large negative inpacts on ecosystems and aquaculture in both brackish and marine waters. Recently, local blooms of this genus have been observed in the Baltic Sea area. It is currently under question if the high natural organic load into the Baltic Sea basin might partially explain the occurance of these toxic blooms. The hypothesis that *P. patelliferum* , isolated from the Swedish west coast, could use the nitrogen from freshly extracted riverine dissolved organic material (DOM; MW > 1000), either directly or indirectly via bacteria was tested in discontinuously diluted cultures at a dilution rate of 0.3 d-1. Five different ratios of nitrate and riverine dissolved organic nitrogen (DON) (1, 0.75, 0.5, 0.25 and 0) were supplied at a total nitrogen concentration of 20 μM. Axenic as well as non-axenic cultures of the same strain were tested. However, in the course of the experiment, all cultures were infected by bacteria, probably originating from the DOM extracts. Daily determination of cell density revealed that riverine-DON could not be used efficiently. Moreover, the DOM additions caused a small negative effect on *P. patelliferum* cell number. Possibly, this is caused by nitrate uptake by the bacteria growing on the DOM. A direct negative effect of bacteria growing on the organic material on the growth of *P. patelliferum* could not be excluded. This last hypothesis is supported by the fact that in batch culture, axenic cultures showed a higher maximum specific growth rate than non-axenic cultures.The toxicity of this strain, measured as hemolytic potential, was independent on the DOM additions to the cultures.

GROWTH AND DOMOIC ACID PRODUCTION BY *PSEUDO-NITZSCHIA MULTISERIES* AND *P. AUSTRALIS* UNDER NITRATE, SILICATE, AND PHOSPHATE LIMITED CONDITIONS.

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Several studies have shown that domoic acid production by *Pseudo-nitzschia* species varies as a function of the limiting nutrient. Generally there is a requirement for nitrogen replete conditions for domoic acid production, with both phosphate and silicate limitation resulting in production of the toxin. Most studies have focused on *P. multiseries*. The present study focuses on a comparison of growth rates and toxin production between both *P. multiseries* and *P. australis,* the species responsible for two major toxic blooms in Monterey Bay within the last nine years. Clones of *P. multiseries* and *P. australis* were isolated from Pacific coastal waters during the fall of 1998. *P. multiseries* and *P. australis* were grown in batch culture under continuous saturating light (120microEinsteins per meter squared per second) at 14 C. Guillard's f/2 formulation was modified to produce nitrate-, silicate- and phosphate-limited media. Standard f/2 was used as an additional treatment for more direct comparison with previous studies using highly enriched media. Experiments were conducted with four replicates for each treatment and the control. Samples were taken daily for cell counts, particulate and dissolved domoic acid (DA), and dissolved nutrients. Upon cessation of exponential growth, two replicates for each treatment were spiked with the limiting nutrient in an amount equal to the initial limiting nutrient concentration. The stationary growth phase was reached between days 9 and 13 depending on treatment and species. Experiments were maintained for an additional 15 to 20 days after the nutrient spike. Domoic acid was analyzed using FMOC derivitization followed by HPLC analysis (i.e., Pocklington et al. 1990).

Preliminary results suggest that although P. multiseries generally grows more rapidly than P. australis, when compared by volume their toxin contents are not significantly different. Comparative data for toxin production concomitant with nutrient depletion will be shown for both species after analyses of domoic acid and nutrients are completed.

DINOPHYSISTOXIN-1 AND ESTERIFIED DINOPHYSISTOXIN-1 IN THE MUSSEL *MYTILUS GALLOPROVINCIALIS* FED ON TOXIC DINOFLAGELLATE *DINOPHYSIS FORTII*

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Non-toxic mussels *Mytilus galloprovincialis* were fed for 5 days with a natural population of the toxic dinoflagellate *Dinophysis fortii.* The total number of *D. fortii* cells ingested by five mussels during the feeding experiment was approximately 234 x 10(3). Okadaic acid (OA), dinophysistoxin-1 (DTX1) and esterified toxins of OA and DTX1 in D. fortii and mussel extracts were determined by liquid chromatography followed by on-line atmospheric pressure electrospray ionization-mass spectrometric (ESI-MS) detection. DTX1 was the only derivative detected in extracts of the *D. fortii* cells used for the feeding experiment whereas mussels fed on *D. fortii* contained both DTX1 and esterified DTX1. DTX1 was present in significantly higher amounts in the mussels than esterified DTX1. The absorption efficiency of DTX1 in the midgut glands of mussels was approximately 9 % of the total amount of DTX1 in *D. fortii* cells filtered by the mussels. The absorption efficiency is higher than that reported previously in scallops (less than 3%).

RESISTANCE OF MUSSEL (*MYTILUS EDULIS*) BLOOD CELLS TO CYTOTOXIC EFFECTS OF OKADAIC ACID

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Mussel blood cells were incubated for 24-72 h in the presence of 10 nM-1 &μM okadaic acid (OA). The viability was measured using EOSIN Y exclusion. In 1 μM OA, blood cells showed a decreased viability after 48 h (73%) and 72 h (54%) compared to control cells (90% and 88% respectively). No reduction in viability was detected in 10 and 100 nM OA compared to control cells. We proposed that the resistance to cytotoxic effects of OA were due to p-glykoprotein activity (Multi-Drug Resistance) in the blood cell membranes, reducing the intracellular concentration by transporting OA to the surrounding medium. To test predictions about the function and activity of p-glykoproteins in mussel blood cells, we used two known substrates (Rhodamine B and Vincristine) and inhibitors (Verapamil and Staurosporine) of p-glykoprotein activity. In contrast to previous findings, verapamil and staurosporine did not block the outflux of the substrates. Instead, intracellular concentrations of both substrates decreased in the presence of the inhibitors in several repeated experiments. To explain these results, we suggested that the main site for p-glykoprotein activity is in the lysosomal membranes where substrates can be transported from the cytosol into the lysosomal compartments. The volume of the lysosomal compartment was measured using uptake of Neutral Red in cells that had been exposed to different concentrations of OA. Compared to controls, increased lysosomal volume was detected in 10 and 100 nM OA (27% and 50% respectively) but to a lesser extent in 1 μM OA (11%). This suggests that OA affect the lysosomal system, possibly by accumulating within the lysosomes. We propose that the granular mussel blood cells, having a highly developed lysosomal system, can accumulate high concentrations of toxic substances such as OA Thereby, the lysosomal system protects the blood cells from potential toxic effects of OA.

IRON NUTRITION IN THE BROWN TIDE ORGANISM, *AUREOCOCCUS ANOPHAGEFFERENS*

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Blooms of the Pelagophyte, *Aureococcus anophagefferens* are responsible for the brown tides that occur in the Peconic estuary on Long Island, NY. To understand the role of iron in controlling these blooms, the growth of *A. anophagefferens* was examined in artificial seawater supplemented with f/2 nutrients using trace metal clean techniques. Two different conditions of iron availability were utilized; (1) iron was complexed with twice its concentration using EDTA or NTA, and (2) cultures were grown in trace-metal buffered media using 100 μM excess EDTA. Cultures grown on 117 nM added iron (total Fe concentration = 140 nM) gave maximum cell numbers (9 x 10e9 cell / L), maximum fluorescence (~ 850), and growth rate (~1 div per day) similar to that obtained in iron-replete (11 μ;M) cultures. Thus the iron-quota for *A. anophagefferens* was lower than 100 nM. Even at 0 μM added iron, the maximum cell yield of *Aureococcus* was not significantly different from the 11 μM iron-replete controls. The use of trace-metal buffered conditions did not significantly affect the growth of *A. anophagefferens* in culture. The estimated iron quota (QFe) for these cultures, <5 mol/cell, is in agreement with the possible oceanic origin of these cultures and the observed drop in iron concentration during bloom formation. To understand how *A. anophagefferens* obtains its needed iron, the enzyme ferric chelate reductase was characterized from this organism. This constitutive enzyme shows a broad pH optimum, is bispecific for NADPH (preferred) and NADH, and has a Vmax (0.57-1.3 μmol/min/mg protein) and Km (370-900 μM) for chelated iron typical of most eukaryotic algae. The observed rates of enzyme activity (average 20 μmol/min/mg protein using NADPH) are sufficient to support the maximum growth rate of the organism. Laboratory cultures showed no evidence for siderophore or chelator formation under low iron conditions. We conclude that *A. anophagefferens* CCMP1708 has a very low iron requirement and the necessary mechanisms to obtain that iron. It is unlikely that this trace metal would limit bloom formation. This work was supported by New York State Sea Grant and the Suffolk County Department of Health.

A NEW HARMFUL DINOFLAGELLATE OCCURRED IN COASTAL WATERS OF WEST JAPAN

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Blooms caused by a new unarmored-dinoflagellate (*Gyrodinium* sp.) has been occurred in the coastal waters of west Japan since 1995. The cell of this species is spherical to ovoid and almost circular in transverse cross section. Cells are 17-23 micro-meter long and 13-18 micro-meter wide. The cingulum is median and encircles equatorially around the cell. The both cingular ends are displaced about twice cingulum widths or 0.2-0.25 of the cell length. The short and narrow sulcus slightly invades onto the epicone. The sulcus reaches to the antapex. It is curved to the left at the under intersection with the cingulum and forms a small embayment. The linear apical groove is sculptured on the epicone. It emerges from just above the proximal end of the cingulum, and reaches at the halfway of dorsal side of the epicone. The groove is rather wide at both ends, but it is gradually narrowed to the apex. The nucleus is large, spherical to ovoid, and is mainly situated in the hypocone. Chloroplasts are yellowish brown and irregular in shape, and usually ten to twenty in number. Even in low cell density this species showed very strong ichtyotoxicity, which continued for a week after passing the bloom. The scales of all fishes killed in the bloom were stood on end and the skins of them were whitened. The skins and gills of fishes damaged severely were festered and redden with blood. Also, the cell of lavers(*Porphyra tenera*), which were cultured in the area suffered by the red tide, grew irregularly. It seems that this species have some substance which attack to not only animals but also plants.

DECONTAMINATION RATES OF PSP IN SHELFISH FROM OUALIDIA LAGOON IN MOROCCO

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During late 1999, an outbreak of PSP occurred along the Ibero ˆ Moroccan shores. It was associated with massive bloom of *Gymnodinium catenatum*. Thus, a continuous surveillance plan was carried out in the interested area. The frequency of sampling was intensified and the analyses were carried out by AOAC mouse bioassay. According to the results from the surveillance of PSP toxins during this campaign, reliable information on toxicity of shellfish was obtained from the monitoring of toxins in various species of shellfish. This results indicate there are substantial differences in toxin level among three populations of shellfish namely mussel (*Mytilus galloprovinciallis*), oyster (*Crassoteria gigas*) and clam (*Redutapes decussatus*) living in the same area in Oualidia lagoon. There was also a large variation of PSP toxins accumulation and detoxification for the three species of shellfish mentioned above.

OKADAÏC ACID AND DINOPHYSISTOXINE-2 IN MOROCCO

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Since 1997 the Moroccan bivalves started being monitored for DSP toxins using the mouse bioassay method of Yasumoto 1984 and 1987. The aim was to be able to advice people on the risk associated with eating wild shellfish. At first time in summer period of 1999 the positive results were detected in mussels. So the identification by HPLC/MS revealed the presence of Okadaïc acid and Dinophysistoxin-2 in high amounts.

IN SITU DATA AND REMOTE SENSING ANALYSIS: A RED TIDE CASE STUDY IN HONG KONG WATERS

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Harmful algal blooms have significant and growing impacts on human health and fisheries resources throughout the word. In recent years, the increasing number of red have tides posed a serious threat to the fishing and fish culture industry in the coastal of China Chinese seas including Hong Kong waters. This study analyzes both satellite (NOAA and SeaWiFS) remote sensing data and in situ seawater measurements of a red tide that occurred at the mouth of the Pearl River (the northern region of the South China Sea) on 17 November 1998. A multi-parameter environmental sensor system was used to obtain real-time measurements of critical water quality properties. The red tide cells concentration reached 3.8 millions per liter. They appeared as many parallel trips 100 to 500m long and 10 to 20 m wide. The total area of the red tide was about 50 km2 near Macao (113'38.165 E, 22'09.691 N). The species of red tide is still being identified. Chlorophyll-a concentrations was very high (250 volts); the surface oxygen saturation was 120-126 (%); and the surface temperature was 24.5 – 25 oC. Results show that the nutrients originated from river discharge water with low salinity (17 to 19 ‰), and the water was very stratified when the red tide occurred.

PATTERNS OF SELECTIVE FEEDING ON *ALEXANDRIUM* SP. BY ZOOPLANKTON IN THE GULF OF MAINE

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Zooplankton may feed selectively, and the ability of some copepod species to detect and avoid consuming toxic prey has been demonstrated in the laboratory. To test whether such selective feeding is practiced under more natural conditions, experiments were performed in 1998 and 1999 with wild zooplankton and natural water samples during the spring population increase of the dinoflagellate *Alexandrium fundyense* in the Gulf of Maine. The copepod *Acartia hudsonica*, a dominant coastal/inshore zooplankton species, did not discriminate against *A. fundyense,* consuming toxic cells at rates equivalent to those on other phytoplankton throughout the study. Barnacle nauplii *(Semibalanus* sp.) consistently avoided consumption of *A. fundyense*, preferring small diatoms such as *Chaetoceros socialis*. The copepod *Calanus finmarchicus,* a zooplankton dominant in offshore waters, avoided consumption of *A. fundyense* and other dinoflagellates when diatoms such as *C. socialis* were abundant, but consumed *A. fundyense* along with other dinoflagellates and ciliates when diatoms were scarce. Zooplankton avoidance of *Alexandrium* spp. early in the season may enhance bloom formation, but the observed variability of feeding behavior among zooplankton species supports previous hypotheses that mortality of *Alexandrium* spp. populations due to grazing will depend on the composition of the grazing community.

ASSIMILATION AND RETENTION OF PSP TOXINS BY ZOOPLANKTON GRAZERS, WITH IMPLICATIONS FOR THEIR ROLE AS TOXIN VECTORS

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Zooplankton are known to act as vectors of phycotoxins to higher trophic levels, such as fish and marine mammals. To assess the potential magnitude of this threat, we performed experiments to determine the efficiency of PSP toxin assimilation by copepod grazers that commonly co-occur with the toxigenic dinoflagellate *Alexandrium fundyense* in coastal waters of the Gulf of Maine. *Acartia hudsonica, Centropages hamatus*, and *Eurytemora herdmani* were fed cultured *A. fundyense* cells, as a monoculture and also in mixtures with the non-toxic dinoflagellate *Heterocapsa triquetra.* Toxin levels in the *A. fundyense* prey, copepod tissues, and fecal pellets were analyzed by high-performance liquid chromatography with fluorescence detection (HPLC-FD). Feeding rates were variable and moderate (20-80% body carbon day-1). In all cases, efficiency of toxin assimilation and retention in tissues (ingested/retained) was very low (<5%). Fecal pellet production was also low, and analysis of fecal material showed toxin levels similar to values in copepod tissue (<5% of toxin ingested). Experiments measuring copepod growth likewise showed poor carbon assimilation (either no growth or body carbon losses) when fed toxic *Alexandrium* sp., despite moderate to high feeding rates, whereas non-toxic *Alexandrium* sp. supported growth at high rates (up to 20% body carbon day-1). The poor assimilation of both carbon and PSP toxins by various copepods when fed *Alexandrium fundyense*, and low fecal pellet production, implies that most "ingested" material is discarded rather than digested (e.g., via sloppy feeding or regurgitation). Although zooplankton may accumulate toxins to levels that are deleterious to higher trophic levels, low retention efficiencies of copepods, in particular, suggests that they are inefficient vectors of PSP toxins.

PHYLOGENETIC ANALYSES INDICATE THAT THE 19`HEXANOYLOXY-FUCOXANTHIN-CONTAINING DINOFLAGELLATES HAVE TERTIARY PLASTIDS OF HAPTOPHYTE ORIGIN

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The three anomalously pigmented dinoflagellates *Gymnodinium galatheanum, Gyrodinium aureolum* and *Gymnodinium breve* have plastids possessing 19`-hexanoyloxy-fucoxanthin as the major carotenoid rather than peridinin which is characteristic of the majority of dinoflagellate plastids. Analyses of ssu rRNA from the plastid and the nuclear genome of these dinoflagellate species indicate that they have acquired their plastids via endosymbiosis of a haptophyte. The dinoflagellate plastid sequences appear to have undergone rapid sequence evolution, and there is considerable divergence between the three species. However, distance, parsimony and maximum likelihood phylogenetic analyses place the three species within the haptophyte clade. *Pavlova gyrans* is the most basal branching haptophyte, and is the outgroup to a clade comprised of the dinoflagellate sequences and those of other haptophytes. The haptophytes themselves are thought to have plastids of a secondary origin, hence these dinoflagellates appear to have tertiary plastids. Both molecular and morphological data divide the plastids into two groups, where *Gyrodinium aureolum* and *Gymnodinium breve* have similar plastid morphology and *Gymnodinium galatheanum* has plastids with unique features.

AN OVERVIEW OF THE BIODIVERSITY OF BENTHIC DINOFLAGELLATES FROM LA REUNION ISLAND (FRANCE, SOUT-WEST INDIAN OCEAN)

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This study examined the distribution of toxic and non-toxic benthic thecate dinoflagellates from La Réunion Island, France (SW Indian Ocean). We report on the diversity and ecology of 29 taxa of benthic dinoflagellates in coral reef or sediment habitat at three representative sites. 15 benthic thecate dinoflagellate species, considered harmful, are observed in La Réunion Island: *Gambierdiscus toxicus, G. yasumotoi, Ostreopsis heptagona, O. lenticularis, O. mascarenensis, O. ovata, O. siamensis, Coolia monotis, Prorocentrum arenarium, P. belizeanum, P. concavum, P. hoffmanianum, P. lima, P. mexicanum* and a new species of *Prorocentrum*. In addition, two potential toxic species, *Coolia tropicalis* and a new *Coolia* species, are also observed. Seven toxic species are from the genus *Prorocentrum*. Dinoflagellate biodiversity in La Réunion Island is illustrated by scanning electron microscope study and the distinct dinoflagellate assemblage between sampling sites is discussed. With this complementary approach in the knowledge of tropical dinoflagellates, it appears that, for a good understanding of the tropical toxic outbreaks caused by dinoflagellates toxins, it is necessary to have a good knowledge of the benthic dinoflagellates both in coral reef and sandy ecosystems, particularly in the western Indian Ocean where new types of seafood poisoning.

COASTAL PHYTOPLANKTON RESPONSES TO A LARGE POINT SOURCE OF NUTRIENTS

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Since March 1996 the phytoplankton biomass, species composition and primary production have been measured in the vicinity of a wastewater outlet near the city of Perth, Western Australia. The wastewater discharge delivers some 2,518 kg of nitrogen and 683 kg of phosphorous per day into the oligotrophic marine waters of Ocean Reef some 1.7 km from shore. Within the vicinity of the outlet there were measurably greater concentrations of nitrate, phosphate and silicate. The low salinity of the wastewater means the effluent rises to the surface. Under relatively calm conditions there was a measurable phytoplankton response in the vicinity of the outlet. Phytoplankton biomass and productivity are some 50% greater up to 4 km away from the outlet. The ecosystem was strongly dominated by diatoms, which made up ~ 55% of all phytoplankton cells. Diatoms actually increased their dominance in the vicinity of the wastewater outlet. This fact may be related to the unusually low levels of dissolved inorganic nitrogen relative to phosphate and silicate in the ambient seawater. For most of the summer the measured N:P ratio in the ambient seawater is ~ 1:1, far below the optimum 16:1 for phytoplankton growth. Unlike most wastewater discharges in this environment the wastewater raises the N:P ratio and increases the silicate concentrations. Phytoplankton biodiversity was not impacted by the nutrient discharge. Phytoplankton taxa considered problematic in this ecosystem, silicoflagellates (mostly *Dictyocha octonaria*) and Cyanophyta (largely *Oscillatoria erythraea*), appeared only sporadically and were no more prevalent in the vicinity of the wastewater outlet than at the control site.

THE OCCURRENCE OF PARALYTIC SHELLFISH TOXINS IN SHELLFISH FROM DAYA BAY AND DAPENG BAY, GUANGDONG

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From Oct. 1997-June 1999, the paralytic shellfish poisonin (PSP) toxicity levels of scallop *Chlamys nobilis* and blue mussel *Perna viridis*, which were collected monthly from four shellfish growing zones in Daya Bay and Dapeng Bay, were determined with the AOAC bioassay method. The results showed PSP was accumulated to a difference levels in the two species and four zones. High toxins levels have been detected in scallop samples from Dongshan station in Daya Bay, from 1263000 to 342000Mu/100g tissue for ingest gland , and from 13400 to 5300Mu/100g tissue for whole tissue excluded ingest gland, and the levels of whole tissue were from 117 to 29.5 Mu/100g tissues during January to May 1999, the toxin levels of whole samples extremely exceeded the public health safety threshold ,but the levels of blue mussel samples from the same station were much less than scallop ,from 567000 to 3100 Mu/100g tissue and from 14200 Mu/100g tissue to undetected for ingest gland and whole tissue excluded former respectively, the levels of whole tissue were from 72800 Mu/100g to undetectable and only one sample`s level was beyond the threshold in January. The levels gradually decreased monthly since winter-spring season . In Aotao , another samples station in Daya Bay, the levels of two shellfish species were lower than the threshold and undetected for most mussel samples . The toxins reached maximum levels at the station during May 1998. Less toxin levels were also detected in the shellfish cultured in Dapeng Bay, however toxins have persisted at lower , still exceeding the threshold , in some scallop ingest gland samples, Relationship among the toxin levels and shellfish species , oceanography , seasonal change was discussed.

TEMPORAL EVOLUTION OF PHOTOSYNTHETIC PARAMETERS AND PRIMARY PRODUCTION DURING A BLOOM OF AN ICHTHYOTOXIC PRYMNESIOPHYTE UNDER DIFFERENT N / P RATIOS.

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A mesocosm experiment was conducted on a toxic Prymnesiophyte of the genus *Chrysochromulina* under different N / P regimes. Many species within the genus are photosynthetic and can produce ichthyotoxic substances especially under phosphate deficient conditions, which can be lethal to fish and invertebrates. The temporal evolution in chlorophyll a, photosynthetic parameters (light saturated slope , maximum photosynthetic rate , maximum quantum yield , the light saturation parameters EK(PAR) & EK(PUR)), light absorption spectra of phytoplankton, fluorescence excitation spectra and primary production were studied over 18 days to assess the correlation between nutrient regime, photosynthetic activity and primary production. 8 mesocosms with N / P ratios ranging from 0.8 to 320 were sampled every 3 to 4 days. Diel cycle experiments of the variation in photosynthetic parameters were also conducted. Preliminary results suggest that low N / P ratios adversely affect photosynthetic activity and primary production, which could also modulate toxin production. The results are discussed in terms of changes in bio-optical and photosynthetic properties of the Prymnesiophyte under different N / P ratios.

THE DEVELOPMENT OF A NATIONAL HARMFUL ALGAL DATA MANAGEMENT SYSTEM

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The US National Oceanographic Data Center (NODC) of NOAA is developing a system which will provide access to physical, chemical, and biological information acquired from various sources, to assist in harmful algal bloom (HAB) management and research. Initially, a prototype system will be developed for the Gulf of Maine and the Gulf of Mexico in coordination with the Ecology and Oceanography of Harmful Algal Bloom (ECOHAB) program. This system will be expanded to include data from other US coastal areas where HABs occur. Sources of data include routine monitoring efforts, event driven monitoring, topical research initiatives (ECOHAB), and the NODC archive. Monitoring and biological data types will be held in a new database at the NODC. A common interface will link this database to other sources of HAB-related data such as the NODC's Ocean Profile Database and Ocean Current Time Series Database. This data management system will allow researchers and resource managers to access data in a particular region to better understand the dynamics that cause the occurrence and decline of bloom events. Physical, chemical, and biological data from many sources will be provided via this system to support research and modeling efforts, as well as coastal resource management.

AN ELISA-BASED SCREENING SYSTEM FOR USE IN REGULATORY MONITORING OF SHELLFISH BIOTOXINS

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In New Zealand , the aquaculture industry must test for the presence of ASP, DSP, NSP and PSP toxins in harvested shellfish because all four toxin groups have been found at levels above maximum permitted limits (MPL) at some time in the past 5 years. To reduce the costs of regulatory shellfish testing, the industry, and NZ regulatory authority have determined that it would be more cost efficient to introduce a screening test to identify shellfish which contain toxin levels approaching the MPL. To date, most other countries test for only one or two of the toxin groups, most commonly PSP and DSP, however the with the recognition of toxic algal blooms increasing around the world, this may become a cost-efficient scheme for use world-wide. An industry-wide working group proposed the following operating parameters for a screening system: (i) a single simple extraction procedure; (ii) minimum sample size consistent with representative sampling; (iii) extraction and analysis to capture all four toxin groups; (iv) Yes/No answer for toxin presence; (v) no false negatives; (vi) no use of animals (i.e. no mouse bioassays); (vii) fast results (<20 hrs) with high throughput (35/day); (viii) applicable to all commercial shellfish; (ix) cost effective. We have developed ELISA for selected ASP, NSP, and DSP toxins and for YTX, and employed these assays along with ELISAs for STX and neo-STX (PSP) to establish a common extraction protocol and test the feasibility of such a system. We conclude that an ELISA-based screening system to identify suspect shellfish samples for subsequent analysis by methods approved by international regulatory authorities is feasible, and that the above parameters can be fully satisfied. The ease of ELISA permits the ready expansion of the system to screen for other toxins, as new ELISAs for these toxins become available.

POSSIBLE FACTORS AFFECTING DYNAMICS OF *ALEXANDRIUM* SP. IN THE NORTHERN GULF OF MAINE

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We conducted three surveys of the coastal and offshore waters of the northern Gulf of Maine between New Hampshire and the outer Bay of Fundy during the summer of 1998, collecting data from more than 200 stations during each cruise in June, July and August. Hydrographic data were collected and concentrations of phytoplankton chlorophyll, inorganic nutrients and cell densities of *Alexandrium,* the dinoflagellate responsible for Paralytic Shellfish Poisoning (PSP), were measured in discrete water samples. The distributions of *Alexandrium* on all three cruises displayed maximum cell densities in the offshore surface waters of the Gulf, and not immediately adjacent to the shoreline where PSP toxicity in shellfish is typically reported. Highest cell densities (ca. 5,500 per liter) were observed in two broad patches: one in the Bay of Fundy and another in waters offshore of the central and eastern Maine coast. Examined against satellite images of sea surface temperature, highest cell densities were well correlated spatially with waters having experienced vertical mixing by tides (i.e., the Eastern Maine Coastal Current/Plume system, and western Bay of Fundy waters). As the summer progressed, the highest densities of *Alexandrium* appeared to recede toward the eastern portions of the Gulf and the Bay of Fundy. We suggest that the naturally-occurring offshore distributions of relatively high densities of *Alexandrium* in surface waters can be related to dynamics controlling inorganic nutrient fluxes and the ambient light field as it varies seasonally and vertically. We also suggest that periodic outbreaks of PSP in nearshore waters are caused by wind-driven advection of cells to those shellfish beds.

DOMOIC ACID PRODUCTION IN COASTAL UPWELLING ZONES OFF CALIFORNIA AND OREGON, U.S.A., ASSOCIATED WITH TOXIFICATION EVENTS

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The first confirmed death of a marine mammal due to domoic acid poisoning was reported in California, U.S.A. during May and June, 1998. This mortality of sea lions was determined to be caused by their ingestion of sardines and anchovies which had accumulated high levels of the neurotoxin. Sardines and anchovies which were harvested from nearshore waters between San Francisco Bay and Monterey Bay, California during a 3 week cruise in May showed the highest levels of domoic acid in Monterey Bay (up to 2300 µg DA/g viscera on 24 May), at the same time when sea lions with neurological symptoms stranded in the Bay. Four research cruises off the coasts of California and Oregon were conducted in June and July to determine the distribution of domoic acid-producing *Pseudo- nitzschia.* The pennate diatoms, *Pseudo-nitzschia multiseries* and *P. australis* were the dominant, toxin-producing phytoplankton constituting algal blooms near coastal locations where sea lions with neurological symptoms had stranded, with the highest cellular toxin levels at 6 pg/cell (*P. multiseries*) and 78 pg/cell (*P. australis*). *P. australis* was the dominant *Pseudo-nitzschia* species in nearshore watersoff the central Oregon coast during July, located within 20 km of the coast at a time when domoic acid in razor clams was reported in that same area. Hydrographic data and satellite imagery at the time of these cruises indicated that the greatest numbers of toxic cells were positioned in water masses associated with upwelling zones. Nutrient levels at these sites in California and Oregon were less than those typically measured during periods of active upwelling due to the 1998 El Niûo event. These results agree with long- term studies of phytoplankton succession (Chavez et al., in press) which indicate that pennate diatoms dominate during post-upwelling conditions when nutrient levels are less than maximal.

BIOLOGICAL AND PHYSICAL DYNAMICS OF DOMOIC ACID PRODUCTION OFF THE WASHINGTON U.S.A. COAST

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The relationship among cellular levels of toxin, *Pseudo-nitzschia* distributions, and the energetic and highly variable coastal water masses of an upwelling- dominated region are explored using data collected during two summer cruises off the Washington coast in 1997 and 1998. During both years, an area of maximum domoic acid production was located approximately 50 km off the coast and the stations with the highest domoic acid levels corresponded to high numbers of *Pseudo-nitzschia pseudodelicatissima* (up to 1 million cells/L, 2.5 μg DA/L in 1997 and 200,000 cells/L, 0.2 μg DA/L in 1998), a species confirmed by receptor binding analysis and mass spectroscopy to produce toxin in this region. Other *Pseudo-nitzschia* species were present, but were always less than 5% of the total population when domoic acid was measured. In both years, *Pseudo-nitzschia* cells and domoic acid were observed from 0 to 40 m depth, although the highest levels of cells and toxin were in the upper 20 meters. In 1998, monthly cruises in the summer and early fall indicated that high levels of domoic acid in seawater covered an approximately 100 km2 area of the Washington coast and persisted at least until 1 October. The appearance of high levels of domoic acid (up to 2.6 μg DA/L), coincident with high numbers of *P. pseudodelicatissima* (up to 15 million cells/L) at Kalaloch beach on the central coast in late September, was followed by the accumulation of record levels of toxin in razor clams (287 μg DA/g) approximately one week later. This dramatic increase in toxin-producing cells on the coast immediately followed a strong upwelling event, indicated by high levels of silicate and nitrate in the absence of significant rainfall and hence river flow.

NSP/DSP BIOASSAY MODIFICATION: THE HUNT FOR A SCREEN TEST WHICH DETECTS ALL KNOWN GENUINE SHELLFISH TOXINS BUT NOT GYMNODIMINE

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The shellfish monitoring system in New Zealand relies on the DSP mouse bioassay (using acetone/dichloromethane extraction) to detect the lipid-soluble toxins NSP, DSP, pectenotoxin and yessotoxin. Where bioactivity is detected, further tests are undertaken to identify the toxin responsible. Areas are closed for harvest on the basis of results from these additional tests. The method is becoming increasingly cumbersome as the number of individual toxin tests that are required increases. One of the bioactive molecules most commonly detected in the screen test, gymnodimine, is not orally toxic. If a screen test could be found which did not detect gymnodimine, but did reliably detect all other lipid-soluble toxins, such a screen test could be used to determine whether areas should be available for harvesting, eliminating the need for subsidiary testing. This project aimed to find a screen test method for the lipid soluble toxins which would give negative results with gymnodimine, but positive results with the toxins mentioned above. Several variants of the present acetone extraction method were tried. The most promising was used for limited trials on samples of known toxicity. The method worked well in detecting the genuine toxins but not gymnodimine. However, the number of nontoxic samples which gave positive results in the mouse bioassay increased using this method. The method appeared to give accurate results at the 10 mouse unit (MU) / 100 g level, but gave too many false positives at 5 MU/100 g shellfish. Subsidiary testing for DSP toxins and pectenotoxin at concentrations between 5 and 10 MU/100 g would still be required if this method were to be introduced. It was therefore decided that the benefits of introducing this method were insufficient to outweigh the cost of method validation.

USE OF THE NEUROBLASTOMA ASSAY IN THE DETECTION OF BREVETOXINS, CIGUATOXIN AND SAXITOXINS

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The neuroblastoma assay measures the extent to which the voltage-activated sodium channel is open. Shellfish contamination by saxitoxins and brevetoxins, which act through their effect on these sodium channels, can potentially be measured using this assay. It is an effect-based assay. That is, it measures the overall toxicity, and does not rely on knowing which toxin variants are present. We are evaluating the neuroblastoma assay as a potential replacement for the mouse bioassay in the monitoring programme for New Zealand shellfish. Sample preparation methods and neuroblastoma assays for the determination of brevetoxins and saxitoxins from shellfish are briefly described. The brevetoxin method can also be applied to the determination of ciguatoxins in fish samples. These assays have been applied to a variety of shellfish samples and the results compared with mouse bioassay. The assays were capable of detecting both brevetoxins and saxitoxins present in shellfish at levels below the regulatory limit. Gymnodimine, a sodium channel active compound which is not orally toxic, was not detected in either assay as it was separated from the toxins during sample preparation.Ciguatoxins were detected in three of four imported fish samples where ciguatoxins were suspected of causing illness. Brevetoxins were detected in only two of five supposedly NSP positive samples. Of the remaining samples, two contained sufficient yessotoxin to explain the observed toxicity. The fifth sample contained gymnodimine plus some underlying toxicity. This underlying toxicity was not identified. Saxitoxins were reliably detected in all PSP positive samples tested. The result to date indicate that these assays are suitable to replace the mouse bioassay in the detection of sodium channel active compounds. Further validation work for these assays is in progress.

OKADAIC ACID QUANTIFICATION IN PHYTOPLANKTON USING A PP2A INHIBITION ASSAY

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Algal monitoring programs for Diarrhoetic Shellfish Poisoning (DSP) are generally based on cell counting; however, the quantification of diarrhoetic toxins (DT) in phytoplankton could strongly improve their efficacy. Therefore, we modified a very sensitive PP2A inhibition assay, previously set up to determine DT in mussels (Tubaro et al. 1996), in order to quantify okadaic acid (OA) and its derivatives directly in algal samples. The phytoplankton was collected by vertical net hauls from bottom to surface to concentrate the phytoplankton present in the water column. After centrifugation, the phytoplankton samples were extracted with 80 % methanol, washed with hexane and dried under vacuum. To be submitted to the PP2A inhibition assay, the dried extracts were resuspended with an appropriate amount of methanol. In our conditions the assay was sensitive to 0.08 ng of OA equivalents per L of sample (0.008 ppb). The reproducibility of the assay was good as well as its accuracy: the CV ranged from 1% to 5% and the recovery of OA in "spiked" phytoplankton samples ranged from 102% to 105%. Natural phytoplankton samples, containing different concentrations of *Dinophisis* ssp. (mostly *D.fortii* and *D.caudata*), were analyzed using the modified PP2A inhibition assay. A significant correlation was found between the DT concentration and the *D.fortii* cell number in the sample (r=0.9978; n=4), allowing to calculate an OA equivalent content of about 50 pg /cell. On the contrary, no correlation was found between the toxins content and the *D.caudata* cell number or the total *Dinophysis* cell number. These data confirm the main role of *D.fortii* in DSP contamination in the Gulf of Trieste, Northern Adriatic Sea.

Tubaro A., Florio C., Luxich E., Sosa S., Della Loggia R., Yasumoto T. – Toxicon (1996) 34:743-752.

A RATIONAL STRATEGY TOWARD THE MANAGEMENT OF SEAFOOD POISONING IN THE WESTERN INDIAN OCEAN REGION.

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Numerous seafood poisoning have been identified in the region, involving reef fishes (ciguatera), sharks (carchatoxism), turtles (chelonitoxism), sardines (clupeotoxism), puffer fishes (tetrodotoxism). Carchatoxism is an endemic and severe problem specific of Madagascar. Ecotoxicological processes involved are poorly known (sources and natures of toxins, species involved, ...). These problems being of global concern for the countries of the region and with respect to the requirements of seafood safety for local consumption and external trading, the Regional Environment Program of the Indian Ocean Commission/European Union (REP-COI) established in march 1998 a "Marine Ecotoxicology" component which has focused its activities in the following areas : § Establishment of a regional network based on the national existing facilities of the COI countries, § Capacity building with organization of training workshops, § Survey of risk factors following the 1998 bleaching event, Development of a practical manual for monitoring epidemiological, toxicological and environmental data produced by member of the network. These activities are promoted to meet with the needs of management of coastal ecosystems as part of the Integrated Coastal Zone Management

PHYTOPLANKTON BLOOMS IN A SEASONALLY OPEN BAR-BUILT ESTUARY: BLOOM RESPONSE TO SEDIMENT NUTRIENT EFFLUX DURING SALINITY STRATIFICATION.

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Wilson Inlet is a relatively shallow estuary located on the south coast of Western Australia. In recent years the occurrence of mild spring phytoplankton blooms has raised fears that the system is progressively becoming eutrophic. The factors associated with the initiation and proliferation of the spring phytoplankton bloom in Wilson Inlet were investigated during 1997 and 1998. The spring phytoplankton community dynamics were strongly associated with the opening of the sand-bar, which temporarily closed the estuary thereby restricting exchange with the ocean during most of the autumn and winter. About two weeks after the sand-bar was breached in late winter saltwater entered the estuary as a salt-wedge. Vertical density stratification was established in the deeper estuarine basins where high microbial oxygen demand rapidly deoxygenated the bottom saltwater layer. Subsequently, nutrients formerly bound in the sediments were liberated into the overlying water, thereby elevating the dissolved inorganic nitrogen and phosphorus concentration. The response of the phytoplankton community to the high nutrient concentration was rapid. Nutrient limitation bioassays indicated that during this period the phytoplankton community was more likely to be limited by nitrogen than phosphorus. The uptake of re-cycled inorganic nitrogen (ammonium) was greater than the uptake of new inorganic nitrogen (nitrate) and organic nitrogen (urea). The phytoplankton bloom was short-lived, typically collapsing within two weeks of initiation. The bloom crashed when the nutrient supply from the sediments ceased due to the dispersal of vertical salinity stratification and the return of high oxygen concentration through the water column.

BETTER CONSUMER PROTECTION FOR DSP TOXINS

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DSP human toxication outbreaks with detection in responsible bivalves of very low content of toxins, measured with current methods, have called attention for possible deficience on total toxin extraction. The presence of acyl esters of okadaic acid and DTX2 has been confirmed in several species of Portuguese shellfish by HPLC with fluorometric detection. In certain instances, the content of esters has surpassed 50% of total DSP toxins found. The fluorometric method of Lee, currently used by our laboratory to confirm the presence of DSP toxins, does not detect these toxins. The need to introduce a hydrolysis step in sample preparation for HPLC is thus emphasized due to results obtained. Experiments were also carried out on sample preparation for mouse bioassay.

CELL CYCLE REGULATION IN THE FLORIDA RED TIDE DINOFLAGELLATE, *GYMNODINIUM BREVE*

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The diel cycle is a key regulator of the cell cycle in many dinoflagellates, and may play a rate limiting role in bloom formation. However, the mechanisms by which the diel cycle entrains the cell cycle remain poorly understood. In this study we describe diel phasing of the cell cycle in the Florida red tide dinoflagellate, *Gymnodinium breve* Davis, determine the diel cue which serves to entrain the cell cycle, and provide evidence for the presence of cyclin dependent kinase, a cell cycle regulator which may be responsive to this cue. Four laboratory isolates from the west coast of Florida were compared. When grown on a 16:8 light:dark cycle, all isolates displayed phased cell division, with S-phase beginning 6-8 h into the light phase, and mitosis following 12-14 h later, as determined by flow cytometry. A naturally occurring bloom of *G. breve*, studied over one diel cycle, displayed diel cell cycle phasing similar to that in the laboratory cultures, with S-phase beginning during daylight and the peak of mitosis occurring approximately four hours after sunset. In the laboratory cultures, the dark/light ôdawnö transition was found to provide the diel cue which serves to entrain the *G. breve* cell cycle, whereas the light/dark ôduskö transition did not appear to be involved. Evidence for the presence of cyclin dependent kinase (CDK) in *G. breve* was obtained using two approaches: (1) identification of a 34 kDa protein immunoreactive to an antibody against a conserved amino acid sequence (a -PSTAIR) unique to the CDK protein family and (2) inhibition of the cell cycle by olomoucine, a selective CDK inhibitor. Current work on signaling events which regulate cell cycle progression will be discussed.

FIRST DETECTION OF WIDESPREAD TOXIC EVENTS CAUSED BY *ALEXANDRIUM CATENELLA* IN THE MEDITERRANEAN SEA THOUGH A MONITORING HARBOUR PROGRAM

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Toxic events (both PSP and DSP) in the Mediterranean Sea have only been described in confined waters like bays, lagoons and harbours. *Alexandrium catenella* was first observed in Catalan waters in samples taken for monitoring purpose the summer of 1996 inside the Barcelona harbour. Recurrent blooms were observed the consecutive years during the warm season in this harbour. After its first detection, this species has been recorded in an increasing number of stations along the coast, suggesting a progressive extension of *A. catenella* in the area. Two general and widespread blooms of this species occurred in open near-shore waters the summer of 1998 and 1999 along 100 km of coastline. Those years we checked for PSP toxicity and found that the blooms were toxic (up to 983 μg PSP/100 g mussel meat). The presence of *A. catenella* in Mediterranean waters is discussed in relation to their possible origin in these waters, their extension in the close coastal waters, and also its possible expansion.

HIGH SPATIO-TEMPORAL DETECTION OF HABS IN CONFINED WATERS IN THE NWMEDITERRANEAN: IMPLICATIONS FOR MONITORING PROGRAMS

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The Mediterranean Sea has classically been considered as oligotrophic and shellfish fishing and farming is mainly restricted to confined bays. Consequently, little extensive monitoring efforts have been put in this area and few potentially harmful species have been recorded. However, a more detailed approach to the problem reveals that the most near-shore waters are rich in nutrients. Additionally, in the last decades there has been an increasing tendency to the recreational exploitation of the coastline (e.g. 40 harbours in 400 Km of coast in Catalonia). Each of these harbours is a new micro-ecosystem with reduced turbulence and low water renewal times, favoring dinoflagellate development. Due to the common observation of discolored waters inside the harbours, a systematic sampling program was performed in a large number of harbours and confined bays of the Catalan coast. Data are presented from that systematic program which was performed with weekly periodicity in summer and bimonthly in winter during five years. The main observations are: 1) The presence of high numbers of harmful dinoflagellates, mainly of the genus *Alexandrium* and *Dinophysis*. 2) These potentially harmful species are recorded throughout all the year. 3) Some blooms have been observed to appear recurrently in several stations. 4) Sometimes blooms in the confined stations are coincident with high concentrations of that species in the coastal waters outside the harbour. The implications of this high frequency of HABs detections is discussed in relation to the suitability of the sampling program for the early detection of algal blooms.

THE COASTAL *PSEUDO-NITZSCHIA* FROM THE STATE OF RIO DE JANEIRO, BRASIL

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The occurrence of the diatom genus *Pseudo-nitzschia* has been recorded all along the coast of Brazil (from 2oN to 32oS), observed from close to shore (bays, estuaries, beach surf zones, sand reefs) to shelf and offshore waters. On the coast of the State of Rio de Janeiro (ca. 23oS, 270 km along a east-west coastline) there are three environments for an interesting comparison: Cabo Frio (cape with seasonal upwelling), Guanabara Bay (heavily polluted estuary), Ribeira Bay (pristine waters with little freshwater input besides rainfall). In all these systems, *Pseudo-nitzschia* spp. are a frequent component of the micro-phytoplankton (70-80% of all samples). Cell density can vary from 10^2 cells.l-1 (average concentration in Cabo Frio) to a maximum value of 10^5 cells.l-1 (constant in Guanabara Bay and often found in Ribeira Bay). In Cabo Frio (1973-78), they contributed to the first stages of phytoplankton succession triggered by upwelling, but were also well represented in nutrient-poor, more stratified waters. In Guanabara Bay (1985-87, 1998-99), the occurrence and distribution of this marine genus was mostly determined by salinity gradients: lowest abundances (10^2 cells.l-1) in the inner reaches of the bay and during the rainy season (summer). In Ribeira Bay (1987-97), the homogeneity of local hydrological features allowed for the presence of the genus year around and high relative contributions to the micro-phytoplankton (>50% of cell density). The versatility of this genus, in terms of the variety of environmental settings where it can be found, reinforces the need to understand distributional patterns at the species level, which is in progress based on archived and recently collected samples. At present, six species are identified using light and/or scanning electron microscopy: *P. delicatissima, P. heimii, P. cf. multiseries, P. pseudodelicatissima, P. pungens, P. cf. subfraudulenta.*

PHYLOGENETIC RELATIONSHIP OF *ALEXANDRIUM COHORTICULA* (DINOPHYCEAE) TO OTHER *ALEXANDRIUM* SPECIES BASED ON RIBOSOMAL RNA GENE SEQUENCES

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The phylogenetic relationship of the thecate PSP-toxin producing dinoflagellate *Alexandrium cohorticula* Balech to other species of *Alexandrium* was studied based on nucleotide sequences of the ITS1, ITS2, 5.8S, 18S and 28S subunits of the ribosomal RNA gene cistron. These are the first such sequences available for *A. cohorticula*, which is one of the main producers of paralytic shellfish poisoning toxins in tropical waters. Based on the nucleotide sequences of the 28S, 18S and 5.8S subunits of the rRNA gene, *A. cohorticula* grouped together with *Alexandrium tamarense, Alexandrium catenella* and *Alexandrium fundyense.* More interestingly, *A. cohorticula* was most closely affiliated to *Alexandrium tamarense* isolates from Thailand. This result reaffirmed conclusions from previous studies that, for the *Alexandrium tamarense/ fundyense/ catenella* species complex, geographical origin rather than morphology seems to determine genetic relatedness. The results of this study also suggest that *A. cohorticula* most probably belongs to the same species complex. Ribosomal RNA gene sequences do not seem to be able to separate the PSP toxin producing from the non-producing species of *Alexandrium.*

PSP TOXIN PROFILES IN THE DINOFLAGELLATE *ALEXANDRIUM COHORTICULA* AND TOXIC MUSSELS FROM SEBATU, MALACCA

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The first paralytic shellfish poisoning (PSP) incident in Semenanjung Malaysia was reported in 1991 following the consumption of toxic farmed mussel from Sebatu, Malacca. For some time, the source of the PSP toxins was not known, although it was reported that the toxic dinoflagellate *Gymnodinium catenatum* Graham was present in plankton samples collected from the area. Recently we were able to isolate and grow in batch cultures several clones of the dinoflagellate *Alexandrium cohorticula* Balech from Sebatu. The toxicity of the clones was established through mouse bioassays. The dinoflagellate culture extracts as well as extracts of toxic mussels from the area were also subsequently analysed for PSP toxin profiles by high performance liquid chromatography (HPLC) following the methods of Oshima et al. (1989). The mussel extract contained NEO, STX, dcSTX, GTX1, GTX2, GTX3, GTX4, dcGTX2 and dcGTX3. The dominant derivatives were NEO (21 mole percent), GTX2 (20 mole percent), STX (19 mole percent) and GTX1 (16 mole percent). The same derivatives were found in the dinoflagellate extracts, although the content differed significantly from the mussel extracts. In the dinoflagellate extracts the dominant derivatives were GTX4 (60 mole percent) and GTX3 (30 mole percent). Results from this study showed that the toxin profiles of the dinoflagellates and the toxic mussels matched in composition, which provide strong evidence that the toxins in the mussels originated from *A. cohorticula*. The results also showed that significant transformation of the toxin derivatives could have occurred in the mussels.

AN OVERVIEW OF THE ECOHAB:FLORIDA REGION ON THE WEST FLORIDA SHELF: BASIC HYDROGRAPHIC INFORMATION AND ITS RELATIONSHIP TO A *GYMNODINIUM BREVE* RED TIDE.

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The Ecohab:Florida program conducts monthly quasi-synoptic cruises within an area on the West Florida Shelf (WFS) that extends from Tampa Bay to Ft. Myers. Standard hydrographic measurements are made at 63 stations located on one along isobath transect, one diagonal transect, and three cross-shelf transects. Generally the WFS is considered to be oligotrophic yet blooms of the red tide dinoflagellate, *G. breve*, originate and persist in the region. Several features characterize the Ecohab:Florida area that can be related to bloom formation and persistence: seasonal thermal stratification develops and persists from May through October which leads to elevated near bottom chlorophyll levels potentially fueled by nitrate originating from off-shore deep water; remineralization of these near bottom populations may be a potential source of organic N and P; discharge from two major estuaries which bound the area on the north and south yield a characteristic signal of elevated inorganic and organic nutrients, high chlorophyll within the estuarine plume, and reduced salinity that results in the formation of salinity and thermal fronts during the winter and rainy season, respectively; and ,seasonal wind patterns generate coastal upwelling which may be related to transport and accumulation. This presentation will give an overview of the hydrography of the Ecohab:Florida control volume and offer several hypotheses for bloom inception, growth, persistence, and transport based on the WFS characteristics noted above. Each hypothesis will be examined in more detail in a series of additional presentations with observations on the physiological state, growth, nutrient requirements, and transport of a *G. breve* bloom that started in November 1998 and persisted over a four month period.

ALEXANDRIUM AFFINE (INOE AND FUKUYO) BALECH BLOOM IN AMBON BAY, INDONESIA

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A bloom of *Alexandrium affine* was observed for the first time in Ambon Bay, Indonesia during October – November 1997. The water became reddish brown and covered a portion of the inner part of Ambon Bay (about 10km^2). In one location, the abundance of A. affine was 60 x 10^6 cells l^{-1}, although the average was 2 x 10^6 cells l-1. This outbreak of *A. affine* was unique since this is the first occurrence of an *Alexandrium* bloom reported in Ambon Bay. The bloom occured in the middle of the El-Nino year and there was a significant increase in the sea surface temperature. The influence of river runoff and the amount of rainfall prior to the bloom are suspected to be important factors in maintaining the nutrient level, especially nitrate and ammonium, in Ambon Bay. Prior to the outbreak of *A. affine*, a thick haze resulting from the forest fires from the nearby island had blanketed the Ambon Bay for approximately 2 weeks.

MANAGEMENT IMPLICATIONS OF NUTRIENT FLUCTUATIONS DURING A TOXIC CYANOPHYTE BLOOM IN THE CANNING RIVER, 1997-98

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In summer 1997-98 a toxic bloom of the blue-green algae *Anabaena circinalis* and *Anabaena spiroides* occurred in the Canning River near Perth, WA, contaminating the river and precipitating river closure to the public for bathing and fishing. We execute a simple analysis to determine the important nutrient fluctuations a) leading to the instigation of the bloom and b) leading to bloom termination, in the context of ongoing remediation efforts including artificial oxygenation of the river. We conclude that even at the extremely high maximum concentrations observed (0.25 μg chlorophyll L-1), the cyanophytes had taken up only 15% of the P available to them from sediment efflux during summer anoxia. Evidence suggests the vast majority of the released P (3970 mg m-2) was either left unassimilated in bottom waters or taken up by non-photosynthetic bacteria in bottom waters, and that these waters were isolated from the *Anabaena* spp. bloom by strong temperature stratification. This means that any disruption of stratification by oxygen addition intended as a remediation measure risks creating greater problems of water quality by bringing huge bottom water P stores to the surface with no bacterial sink. Early seasonal oxygenation and / or full prevention of anoxic sediment P release would help cap nutrient release locally and avoid this problem.

INDUCTION OF THE SMALL HEAT-SHOCK PROTEIN, HSP16 IN A TRANSGENIC *HSP16- LACZ* STRAIN OF *CAENORHABDITIS ELEGANS* IN RESPONSE TO EXPOSURE TO EXTRACTS OF CYANOBACTERIA AND MICROCYSTIN-LR.

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Caenorhabditis elegans is a small, free-living hemaphroditic nematode widely used in fundamental research into biochemical and genetic studies of multicellular organisms. We have used the transgenic strain, PC72ubIn5, which is stably transfected with a plasmid encoding a hsp16- *lacZ* fusion gene, to investigate the induction of hsp16 in *C. elegans* following exposure to hepatotoxin- and non-hepatotoxin-containing cyanobacterial cell-free extracts and the purified cyclic heptapeptide hepatotoxin, microcystin-LR. Quantitative assays employed the colorimetric substrate ONPG and qualitative, *in vivo* localisation, the histochemical stain Xgal. Of the 10 cyanobacterial strains tested, all induced significant levels of B-galactosidase activity between 8 and 34 times above that of negative controls. There was no correlation between the level of B-galactosidase activity and the presence of detectable hepatotoxins in the extracts. Induction of B-galactosidase activity was found to be both time- and dose-dependent. With aqueous extracts from *Gloeotrichia* sp., B-galactosidase activity increased rapidly within five hours of exposure, with little further increase in activity found between 7 and 18 hours. A significant increase in activity was found at concentrations above 400 ng dry wt. ml-1. Microcystin-LR was also found to induce B-galactosidase activity at concentrations of 100 ng ml-1. Higher levels of B-galactosidase activity were induced in the presence of extracts of *Gloeotrichia* sp. with solvents of lower polarities. Cellular localisation experiments showed that the *in vivo* localisation of B- galactosidase activity was predominantly in the ovaries and uterus, areas of rapid cell division. Potential mechanisms of induction and the roles of hsp *in vivo* following cyanobacterial toxicosis will be discussed.

SEASONAL VARIATION IN PROTEIN PHOSPHATASE-INHIBITING PHYCOTOXINS IN THE GULF OF RIGA, BALTIC SEA, 1999.

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It has been hypothesised that dissolved organic matter of terrestrial origin is a factor which may contribute to the development of non- siliceous algal-dominated blooms in coastal waters. During the spring and summer of 1999, three cruises (5-10 May; 6-11 June and 26-30 July) were undertaken in the Gulf of Riga, Baltic Sea, with five stations being sampled along a transect from the Daugava River to the open sea. Chlorophyll a concentrations in the upper mixed layer decreased from 10.6 +/-7.09 to 3.84 +/- 0.72 μg L-1 between the first and third cruises. The presence of protein phosphatase-inhibiting toxins was determined by a colorimetric PP1-inhibition assay. These were found predominantly in the surface- and upper mixed layers of the water column. The mean concentrations for the upper layer during the May cruise were 7.72 (maximum 36.2, minimum <<0.1) ng microcystin-LR equivalents L-1 and 37.9 (max. 91.2, min. 3.67) ng MC-LR equivs. L-1 during the July cruise. The presence of nodularin in samples from the third cruise was confirmed by HPLC-DAD analysis. There was no significant correlation between toxin concentration and chlorophyll a concentrations. Correlations between toxin types, concentrations and environmental parameters will be presented and discussed in the context of the hypothesis.

DETECTION OF DSP TOXINS ON THE US WEST COAST

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Dinophysis species have been widely implicated as causing Diarrhetic Shellfish Poisoning in numerous locations on the globe, but DSP has never been reported in the US, even though several *Dinophysis* species, including *D. acuminata, D. fortii,* and *D rotundata*, are common members of North American Pacific coastal phytoplankton communities. We have measured abundances of *Dinophysis acuminata* as high as 19000 cells/l in Monterey Bay, California during the Summer of 1999, suggesting that DSP could occur on the West coast should DSP toxins be present in local Dinophysis cells. We applied a protein phosphatase inhibition assay for okadaic acid and DTX-1 to extracts of phytoplankton tows from Santa Cruz, CA. Results showed a strong correlation between abundance of *Dinophysis acuminata* and PP2a activity inhibition. Preliminary results show DSP toxin activity equivalent to 1.0 pg okadaic acid per cell of *Dinophysis*. To our knowledge, these findings represent the first detection of DSP toxins related to *Dinophysis* on the North American Pacific Coast, and the first indication of toxins in Dinophysis cells detected by PP2a inhibition assay applied directly to phytoplankton material.

IDENTIFICATION OF MECHANISM MODEL ON THE TRENDS OF POPULATION DENSITY OF *PSEUDO-NITZSCHIA PUNGENS* IN DAPENG BAY, SOUTH CHINA SEA

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Based on samples taken from seawater of salt field in Dapeng Bay from March 30th to June 22nd of 1990, 2days interval, the identification of dynamic mechanism model on population growth of *P. pungens* in Dapeng Bay is built. Firstly, Salinity, DIP, Si and Mn are identified as controlling variables of population density of *P.pungens* among the 11 factors like plankton, temperature, salinity, pH, turbidity, dissolved oxygen (DO)., dissolved inorganic nitrogen (DIN), dissolved inorganic phosphate (DIP), Fe, Si and Mn. Secondly, using step-down to the back substitution and introducing auto-regression order into the process of the model identification, a simultaneous regression model derived from six auto-regression and non-linear regression models is established. The result of calculation shows that the fitting degree between simulated and observed values are over 75%.

HISTORICAL BIOGEOGRAPHY OF *ALEXANDRIUM*

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The distribution of the cosmopolitan dinoflagellate *Alexandrium* has been placed within the framework of a monophyletic dispersal hypothesis, with the added complication that human mediated dispersal may have occurred in modern times and confused the natural patterns of morphological and genetic variation. Potential biotic exchange between the Atlantic and Pacific Oceans was possible at low latitudes via the Tethys Sea until the early Miocene, and across the Panama Isthmus until the Pliocene. Subsequent to the closure of these two routes, only trans-Arctic and circum-Antarctic exchanges have been possible. There have been major differences between the two oceans in the Pleistocene and Holocene so far as concerns sea surface temperature regimes, ice conditions, and marine transgressions; these have had major impacts on the evolution of the epipelagic fauna, and it is not possible to understand the present day distribution and diversity of this fauna without taking account of these differences. This paper examines the ecological challenges provoked by these differences, and seeks some possible evolutionary relationships between Atlantic and Pacific representatives of *Alexandrium.*

NUTRITION AND GROWTH KINETICS IN NITROGEN- OR PHOSPHORUS-LIMITED CULTURES OF THE NOVEL RED TIDE DINOFLAGELLATE *HETEROCAPSA CIRCULARISQUAMA*

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Heterocapsa circularisquama is a novel red tide dinoflagellate which causes severe damageto Japanese shellfish aquaculture by killing bivalves. It is worrying that outbreaks of *H. circularisquama* red tide are increasing in intensity and geographical distribution. In order to elucidate the mechanism of red tide outbreaks, nutrition and growth kinetics in nitrogen (N)- or phosphorus (P)-limited semi-continuous cultures were examined. Inorganic N compounds, such as nitrate, nitrite, and ammonium, were found to be good nitrogen sources for the growth, while organic nitrogen (urea and uric acid) was not utilized. *H. circularisquama* was capable of successfully using a wide variety of inorganic and organic phosphorus compounds of different molecular structure as a sole P source. Under N-limited steady state conditions, dilution rate (= growth rate), as a function of cell nitrogen quota, followed the Droop equation. Similarly, dilution rate, as a function of cell P quota, followed the Droop equation under P-limited steady state culture. Kinetic parameters Dm and Kq obtained for N- and P-limited cultures were 1.1 /day and 1.2-1.5 pmol/cell, and 1.1 /day and 89 fmol/cell, respectively. The nutrient availability and kinetic parameters of *H. circularisquama* are compared to other red tide organisms and the ecological implications of these characteristics are discussed.

PREDICTION OF BLOOMS OF TOXIC DINOFLAGELLATES BY EVALUATING ENVIRONMENTAL FACTORS

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Paralytic and diarrhetic shellfish poisonings cause significant damage to coastal bivalve aquaculture in Japan. It is important to predict the occurrence of toxic dinoflagellate blooms, mainly *Alexandrium tamarense* and *Dinophysis fortii*. We extracted the environmental factors which enhanced bloom occurrence using a cross correlation method. The annual average value of cell density and the maximum cell density in the year of the toxic dinoflagellate observed at a station in Ofunato Bay, Iwate Prefecture from 1982 to 1991,were dealt as single data points. The environmental factors examined were wind speed,wind direction, precipitation, sunshine hours and air and sea surface temperature.Moving average values for 10 days, 20 days and 30 days of the environmental factors were dealt as single data points. Correlation diagrams between the toxic dinoflagellate values and the environmental factors were drawn using computer software of a cross correlation method, and the periods when high correlations (0.65) occurred were identified from the diagrams. A regression equation was derived by the multiple regression analysis from the average data of the environmental factors during this period and the toxic dinoflagellate values. These results show that the annual average value of *A. tamarense* was influenced by the amount of precipitation in April and the maximum cell density was also influenced by the precipitation at around 20 days and 150 days before from the day of occurrence of the maximum. The predicted values of *A. tamarense* calculated with the regression equation during 1992 to 1994 were 8.5 times as many as the observed ones. The same procedures were also done for *D. fortii.*

PHYTOPLANKTON BLOOMS IN PORT SHELTER WATERS DURING THE EARLY 1998 RED TIDE IN HONG KONG

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From February to May 1998 there was a continuous algal bloom in the Port Shelter, an inlet of an enclosed bay in the northeast Hong Kong's coastal waters. Phytoplankton data collected from a permanent station located in this area was analysed. Diatoms and dinoflagellates were the two main groups which dominated the phytoplankton. In general, when there was an increase in the density of diatoms there was a decline in the density of dinoflagellate, and vice vera. Dinoflagellate species which included the fish killer *Gyrodinium digitatum*, started to bloom in late February and reached their highest density on March 18, when fish kills were first reported. During the next 16 weeks, dinoflagellate species dominated twice, in mid-February and mid-March. Hydrographic and climatic data suggest that the dinoflagellate bloom might have been triggered by stormy weather and the Kuroshio Current, which flows into Hong Kong from the southeast.

THE OCCURRENCE OF PSP AND DSP CAUSATIVE DINOFLAGELLATES IN NORTHERN VIETNAMESE COASTAL WATER

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In order to detects dinoflagellate possibly responsible for PSP and DSP in northern Vietnamese waters, plankton samples from coastal waters and shrimp culture ponds have been observed. Six *Alexandrium* species, i.e. *A. tamiyavanichii, A. ostenfeldii, A. leei, A. insuetum, A. affine* and *A.* sp. cf. *tamarense*, were found in coastal waters, and *A. minutum* was found in shrimp culture ponds. As cultures of these species could not be established except *A. minutum*, their toxicity could not be studied. Toxin productivity of *A. minutum* was confirmed by HPLC analysis and main toxin compornent are GTX 1, 4. Among *Dinophysis* species that have a potential of DSP toxin production, *D. miles* and *D. caudata* were observed.

A NEW PROTEIN SPECIFICALLY ALGICIDAL TO DINOFLAGELLATES, PRODUCED BY AN ALGICIDAL MARINE BACTERIA

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Some ecological studies on interaction between marine bacteria and algae revealed that algicidal (algal-killing) bacteria might play a significant role in a termination process of algal blooms. We isolated many algicidal bacteria at the end of algal blooms by *Gymnodinium mikimotoi* (Dinophyceae) and *Heterosigma akashiwo* (Raphidophyceae) occurred in nearshore seawaters in Japan. One bacterial strain E401, of which 16S ribosomal RNA sequence indicates it is a new species belonging to genus *Alteromonas* is one of the most strong algicidal bacteria against dinoflagellate. An experiment using a two-chamber cultivation system showed that E401 killed algae through some algicidal substances, and we tried to purify and identify the algicidal substances from the filtrate of E401 *G. mikimotoi* mixed culture. One protein (*G. mikimotoi* killer-1; GMK-1) which molecular weight is about 64kDa was identified as the substance algicidal to five species of dinoflagellates, *G. mikimotoi, G. catenatum,Alexandrium tamarense, A. catenella* and *Heterocapsa circularisquama*. Some marine algae belonging to Raphidophyceae, Prasinophyceae, Euglenophyceae, Ulvophyceae and Bacillariophyceae, were not killed by GMK-1. N-terminal amino acid sequence information suggests that GMK-1 might be a novel protein. Another substance algicidal to *H. akashiwo* was obtained from the filtrate of E401-*H. akashiwo* mixed culture, therefore it is suggested that the algicidal marine bacteria E401 killed a variety of marine algae through various kinds of algicidal substances.

DINOFLAGELLATE CYST RECORDS IN SEDIMENT CORES FROM TWO SITES IN MANILA BAY, PHILIPPINES, WITH DIFFERENT DEGREES OF TOXIC RED TIDE INFLUENCE

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Preliminary analysis of the dinoflagellate cyst assemblages in sediment cores from two sites in Manila Bay, i.e., Cavite, a toxic red tide prone area, and Pampanga, an area with less red tide outbreaks, are presented. At least ten cyst species were present at the Pampanga area, including the main toxic species *Pyrodinium bahamense* var. *compressum*. The cyst assemblage in this area was dominated by *Protoperidinium* spp. followed by *Gonyaulax* spp. *Pyrodinium bahamense* var. *compressum* was also seen in Cavite, which contained cyst species similar to the Pampanga core. However, Cavite appeared to have a higher concentration of dinoflagellate cysts than Pampanga. This difference may be related to differences in productivity, or degrees of eutrophication. However, this could also be a reflection of the diluting effect of greater amounts of sedimentation at Pampanga due to its proximity to the Pampanga River, the largest source of sediment in Manila Bay. Such differences have important implications in toxic red tide management and in choosing sites for mariculture in the Bay.

TAXONOMIC STUDY OF *ALEXANDRIUM* BASED ON THECAL MORPHOLOGY

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Thecal morphology of ca. 20 described species of *Alexandrium* such as *A. tamarense, A. catenella* and *A. minutum* and some undescribed species were observed in details for clarifying their morphological relationship. Plankton samples collected in many locations were used for the study as well as cultures maintained in several culture collections. Among them, several specimens were originated from the type locality. Not only well known species, but rare species, namely *A. tropicale, A. concavum* and *A. satoanum*, were also observed. The observations suggested that the genus could be subdivided into four or five groups by the shape of cells and thecal plates, especially sulcal plates. The present classification system differ from Balech's system, which consists of two subgenera and six groups. Characteristics such as the shape of the posterior sulcal plate, the sixth precingular plate and the position of the anterior attachment pore are good criteria for differentiation of each species. In contrast, some others used conventionally for species criteria, such as the width of anterior sulcal plate, were not useful, because of lack of species specificity. *Alexandrium tamarense* and *A. minutum* might be heterogeneous species, as strain- and area- specific morphological characteristics were observed.

A TRY TO UNDERSTAND HOW NUTRIENTS CONTROL TOXIN PRODUCTION IN *ALEXANDRIUM TAMARENSE*

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A series of experiments (bench, semi-flow-through, turbidstat) was conducted to try to understand how the variation of nutrient (N, P) levels affect the toxin production in a locally (HK) isolated strain of *Alexandrium tamarense*. Bench experiment revealed that toxin contents reached the peak at the beginning of growth log phase and then decrease gradually under the condition of enough N. P. supply. Low concentration of N had minor effects on toxin content at the beginning of log phase but had obvious effect on toxin content to decrease later. Low concentration of P kept toxin level constant and slightly increased later. The ratio of N:P had no obvious impacts on toxin production of the algae in the log phase. Semi flow-through experiments indicated that N limit related to low toxin production and P limit caused high toxin production. The algae produce medium level toxins under turbidstat condition. The mechanism of effects of N and P on toxin production was discussed. The fact that the content of toxins is closely related to the content of Arginine in the algae cells implied that N had impact on toxin production by affecting Arginine level in algae cells directly while P had effect on toxin production through regulating N metabolism in the algae cells.

AN OVERVIEW ON RED TIDE OF SOUTH CHINA SEA, 1998

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Massive harmful algal blooms (HAB) occurred along the coast of Guangdong in 1998. They had significant negative impacts on the society. Causative species in 1998 were very different from the previous outbreaks in the SCS. Some are new recorded, some are rare, some are probably new species, but most are dinoflaglleates. They were: *Phaeocystis globosa* on the eastern part of Guangdong coast, *Gymnodinium mikimotoii* in the mouth of the Pearl River, *Gyrodinium instriatum* in the Shenzhen Bay, *Scrippsiella trochoidea* in Daya Bay.Among the factors that initiate bloom outbreaks, temperature is thought to be one of the most important. Algal bloom occurrence in March and April 1998 apparently coincided with a rise temperature. Nutrients are another key factor linked with algal bloom occurrence. Over loading with nutrients frequently initiate algal bloom. There are also many factors speculated as initiating algal blooms. Among these, 1. climate change, 2. meteorological and oceanographic features, 3. anthropogenic influences are ephasized.

PIGMENT PATTERNS OF TOXIC AND NON TOXIC *PSEUDO-NITZSCHIA* SPECIES (BACILLARIOPHYCEAE).

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Pigment composition of toxic and non-toxic *Pseudo-nitzschia* species were analysed by HPLC. An unexpected pigment diversity was observed in this genus respect to previous studies on pigment composition of marine diatoms. In addition to chlorophyll (Chl) a and minor peaks of MgDVP and a Chl c-like pigment only described previously in the haptophyte *Pavlova gyrans,* three Chl c distribution patterns were observed. The Chl c pigment type, species included in each group and the strains analysed were as follows: (Type I) Chl c1 and Chl c2: *P. multiseries* (CCMP: 1659, 1660, 1712) and *P. australis* (IEO: PS3V, PS5V, PS20V, PS27V, PS28V, PS29V, PS31V, PS34V, PS35V, G1, G2, G4, G5); (Type II) Chl c1 Chl c2 and Chl c3: *P. pungens* (CCMP 1572, IEO- PS30V), *P. delicatissima* (IEO: PS7V, PS8V, PS9V), *P. pseudodelicatissima* (CCMP 1564), and *P. fraudulenta* (IEO: PS2V, PS6V, PS11V, PS15V); and (Type III) Chl c2 and Chl c3: *P. cuspidata* (IEO: PSA1V, F1, F3, PS18V, PS19V). The carotenoid composition was typical for the class Bacillariophyceae: fucoxanthin, diadinoxanthin, diatoxanthin and b,b-carotene. However, cultures analysed at stationary growth phase showed the presence of a carotenoid with lycopene-type spectrum, but less retained than a lycopene standard injected into the HPLC system used, which suggests the presence of one additional oxygenated functional group. The potential utility of such a pigment diversity on the framework of HAB monitoring programs is discussed.

COMPARISON OF SOME PECULIARITIES OF SEASONAL CHANGES OF PHYTOPLANKTON IN THE DIFFERENT REGIONS OF THE BALTIC SEA

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The permanent growing of the phytoplankton biomass of the Baltic Sea during the last 10 years was accompanied by the changes in characters of seasonal vegetation. These changes were expressed also in the structure of phytoplankton community. In eutrophic waters of the Gulf of Finland from May till July 1995 the phytoplankton biomass reached 10 g m^{-3} (May). This level was near that of maximum biomass in 1984 (HELCOM, 1996). From May to July biomass decreased in succession on one order: 10500-1030-150 mg m^{-3}. In May diatoms dominated in abundance (68%) and dinoflagellates in biomass (86%). In June diatoms dominated both in abundance and in biomass (82 and 63% accordingly), in July cyanobacteria were mostly abundant (75 and 44% accordingly). There are 8 potentially harmful and toxic species of cyanobacteria and 5 of dinoflagellates, which are typical in the Gulf of Finland. In the central Baltic (Gotland region) inter-annual trend of biomass was not expressed. In the period from May to July 1995 the biomass alters: 930-138-70 mg m^{-3}. In May dinoflagellates dominated both in abundance and in biomass (68-58%), in June – cyanobacteria (87-56%). The content of Ebriidea (*Ebria tripartita*) was 5-22%. In July dinoflagellates dominated. In the South-West Baltic in summer (June, 1998 and July, 1996) phytoplankton biomass was low (96 and 72 mg m^{-3}). In June diatoms and cyanobacteria dominated in abundance (35 and 33%) and Ebriidea in biomass (49%). In July Ebriidea dominated both in abundance and in biomass (45-61%). In autumn (October, 1997) diatoms developed as usual (1.2 g m^{-3}).

CHANGES IN LIPID COMPOSITION AND MORPHOLOGY OF THE TOXIC DIATOM *PSEUDONITZSCHIA PUNGENS* DURING THE LIFE CYCLE

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Various environmental fluctuations in the sea could control the occurrence of different planktonic algal species and growth stage of algae. Under fluctuating environments in the sea, each algal species is exposed occasionally or even frequently to conditions unfavourable for growth and reproduction. Research of biochemical processes, which may play an important role for the survival in natural environments and the onset of blooms of toxic species are especially actual. The survival and change in lipid and fatty acid composition of toxic diatom *Pseudonitzschia pungens* isolated from the Peter the Great Bay (the Sea of Japan) was investigated. The most of the cells of *P. pungens* obtained from natural assemblages and cultures conformed to the classical description and the lipid composition was found to be typical of diatoms. The lipid and fatty acid composition is shown to vary during the life cycle. The changes observed in the fatty acid composition were due to the change in the proportion of the lipid classes and the increase in the content of polyunsaturated fatty acids, mainly 20:5(n-3). Significant fatty acid changes under conditions of nutrient depletion reflected the progressive changes in the morphology and physiology of the cells. Abundance of abnormal lobed cells were observed in aged culture. During the resting stage the important structural and energy rich lipid compounds were accumulated. It is provides the preparation for the subsequent vegetative stage and outbreak of this species under favourable conditions.

Harmful ALGAL BLOOMS

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