

Marine Science Review - 161

Algae and cyanobacteria, blooms and biotoxins



In this review:

- A. Recent articles – no abstract available
- B. Recent articles with abstracts

A. Recent articles – no abstract available

Llewellyn, L.E. **Saxitoxin, a toxic marine natural product that targets a multitude of receptors.** *Natural Product Reports* 23(2): 200-222, 2006.

Littler, M.M., Littler, D.S., Lapointe, B.E., and Barile, P.J. **Toxic cyanobacteria (blue-green algae) associated with groundwater conduits in the Bahamas.** *Coral Reefs* 25(2): 186, 2006.

Brodie, E.C., Gulland, F.M.D., Greig, D.J., Hunter, M., Jaakola, J., St. Leger, J., Leighfield, T.A., and Van Dolah, F.M. **Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*).** *Marine Mammal Science* 22(3): 700-707, 2006.

B. Recent articles with abstracts

Penna, A., Garces, E., Vila, M., Giacobbe, M.G., Fraga, S., Luglie, A., Bravo, I., Bertozzini, E., and Vernesi, C. ***Alexandrium catenella* (Dinophyceae), a toxic ribotype expanding in the NW Mediterranean Sea.** *Marine Biology* 148(1): 13-23, 2005.

Notes: The presence of the paralytic shellfish poisoning (PSP) dinoflagellate *Alexandrium catenella* in the northwestern (NW) Mediterranean Sea has been known since 1983. From this date on, the species has spread along the Spanish and Italian coastlines. Information concerning *A. catenella* isolates in the NW Mediterranean Sea was gained through phylogenetic studies. Twenty established toxic cultures of *A. catenella* taken from various NW Mediterranean Sea locations were analysed by nucleotide sequencing of the 5.8S rDNA and internal transcribed spacer regions. These rDNA ribosomal markers resulted useful in delineating the phylogenetic position of this species in the genus *Alexandrium* as well as in determining relationships between *A. catenella* isolates from different geographic areas. The phylogenetic position of the Mediterranean *A. catenella* ribotype, when compared to the "*Alexandrium tamarense*/*catenella*/*fundyense* species complex", fits this species complex well. All the Mediterranean *A. catenella* isolates were constituted by only one genetic ribotype. By comparing the isolate sequences with those of other geographic areas, it revealed that the Mediterranean *A. catenella* ribotype was closely related to the *A. catenella* from Japan, Western Pacific Ocean. It was also evident that in temperate Japanese waters, a genetic variability was detected within *A. catenella* isolates; in fact, all strains resulted divergent showing as many as 15 mutational steps. The possibility that *A. catenella* has been recently introduced into the Mediterranean basin from temperate Asian areas is discussed.

Hudnell, H.K. **Chronic biotoxin-associated illness: Multiple-system symptoms, a vision deficit, and effective treatment.** *Neurotoxicology and Teratology* 27(5): 733-743, 2005.

Notes: Blooms of toxigenic organisms have increased in spatial and temporal extent due to human activities and natural forces that alter ecologic habitats and pollute the environment. In aquatic environments, harmful algal blooms pose a risk for human

health, the viability of organisms, and the sustainability of ecosystems. The estuarine dinoflagellate, *Pfiesteria piscicida*, was discovered in the late 1980s at North Carolina State University as a contaminant in fish cultures. *P. piscicida* was associated with fish death in laboratory aquaria, and illness among laboratory workers who inhaled the mist above aquaria. Both the fish and humans exhibited signs of toxicity. During the 1990s, large-scale mortality among fish and other aquatic organisms was associated with high concentrations of *Pfiesteria* sp. in estuaries on the eastern seaboard of North America from New York to Texas. Illness among humans was associated with direct exposure to estuaries and exposures to estuarine aerosols around the time of *Pfiesteria*-related fish kills. This review of the scientific literature on associations between *Pfiesteria* and human illness identified some of the possible mechanisms of action by which putative *Pfiesteria* toxins may have caused morbidity. Particular attention was given to the *Pfiesteria*-associated, human-illness syndrome known as Possible Estuary Associated Syndrome (PEAS). PEAS was characterized by multiple-system symptoms, deficits in neuropsychological tests of cognitive function, and rapid and severe decrements in visual contrast sensitivity (VCS), an indicator of neurologic function in the visual system. PEAS was diagnosed in acute and chronic illness cases, and was reacquired during re-exposure. Rapid normalization of PEAS signs and symptoms was achieved through the use of cholestyramine therapy. Cholestyramine, a non-absorbable polymer, has been used by humans to lower cholesterol levels since it was approved for that use by the U.S. Food and Drug Administration in 1958. When dissolved in water or juice and taken orally, cholestyramine binds with cholesterol, bile acids, and salts in the intestines, causing them to be eliminated rather than reabsorbed with bile during enterohepatic recirculation. Cholestyramine also has been reported to bind and eliminate a variety of toxic substances. The efficacy of cholestyramine therapy in treatment of PEAS supported the hypothesis that PEAS is a biotoxin-associated illness.

Jorgensen, K., Scanlon, S., and Jensen, L.B. **Diarrhetic shellfish poisoning toxin esters in Danish blue mussels and surf clams.** *Food Additives and Contaminants* 22(8): 743-751, 2005.

Notes: Until recently, little focus was given to the presence of diarrhetic shellfish poisoning (DSP) toxin esters in seafood products. However, during the last few years, the occurrence of a high percentage of esters of the total amount of DSP toxins present in some seafood products has been observed. Samples of Danish surf clams (*Spisola* spp.) and blue mussels (*Mytilus edulis*) from 1999 - 2004 were analysed by liquid chromatography coupled with tandem mass spectrometry (LC/ MS/ MS) for the presence of DSP toxin esters. The samples contained only okadaic acid and esters of okadaic acid. The level of total okadaic acid equivalents ranged from 224 to 2516 $\mu\text{g kg}^{-1}$ in surf clams. The percentage of okadaic acid esters of the total okadaic acid equivalents ranged from 83 to 98%, mean 95%. The level of total okadaic acid equivalents ranged from 43 to 1631 $\mu\text{g kg}^{-1}$ in blue mussels. The percentage of okadaic acid esters of the total okadaic acid equivalents ranged from 21 to 86%, mean 59%. The probability of a high percentage of okadaic acid esters seems to increase with higher amounts of total okadaic acid equivalents in the bivalves. The large prevalence of DSP toxin esters are of particular importance because of the increased use of chemical methods instead of mouse bioassay for the detection of DSP toxicity.

Gobler, C.J., Lonsdale, D.J., and Boyer, G.L. **A review of the causes, effects, and potential management of harmful brown tide blooms caused by *Aureococcus anophagefferens* (Hargraves et Sieburth).** *Estuaries* 28(5): 726-749, 2005.

Notes: Brown tides caused by the harmful alga *Aureococcus anophagefferens* abruptly appeared in some coastal embayments of the northeastern United States (Rhode Island, New York) in 1985. Since then, brown tides have vanished from some bays, chronically reoccurred in others, and recently have exhibited an apparent southern expansion into new regions (e.g., New Jersey, Delaware, Maryland, and Virginia). Brown tides have also recently been detected across the Atlantic Ocean in South Africa. Although blooms of *A. anophagefferens* have no known direct, negative effects on human health, they are considered harmful because of their detrimental effects on estuarine organisms, such as suspension feeders (scallops and hard clams) and submerged aquatic vegetation. The selective effect of blooms on pelagic grazers (zooplankton and shellfish) is likely to affect food webs and biodiversity within affected ecosystems. Recent findings indicate brown tides occur in shallow estuaries with long residence times and high salinities (> 25 parts per thousand). These estuarine characteristics may foster the accumulation of algal biomass and a nutrient environment (high dissolved organic matter and low dissolved inorganic nitrogen) as well as a low light regime that encourages rapid cellular growth of *A. anophagefferens*. A lack of sufficient grazing control by benthic and pelagic suspension feeders during the initiation phase of blooms is also implicated in brown tide development.

Costa, P.R., Rosa, R., Pereira, J., and Sampayo, M.A.M. **Detection of domoic acid, the amnesic shellfish toxin, in the digestive gland of *Eledone cirrhosa* and *E. moschata* (Cephalopoda, Octopoda) from the Portuguese coast.** *Aquatic Living Resources* 18(4): 395-400, 2005.

Notes: Domoic acid (DA), the toxin responsible for the illness known as amnesic shellfish poisoning (ASP), is an algal toxin produced naturally by some species of the diatom genus *Pseudo-nitzschia*. The toxin has been detected in a diverse array of marine organisms from copepods to whales. Cephalopods, which are important members of the food chain and active predators of known toxin vectors such as bivalves, crabs and some fishes, have just recently been implicated in DA transfer or accumulation. Here we present data showing detectable values of DA determined by HPLC-UV (high-performance liquid chromatography and ultraviolet detection) and confirmed by HPLC-MS (mass spectrometric detection) in two octopus species collected along the Portuguese continental coast: *Eledone cirrhosa* and *E. moschata*. Domoic acid was frequently detected in the digestive gland of *E. moschata* and occasionally reached concentrations exceeding 100 mg g⁻¹. In contrast, *E. cirrhosa* contained lower concentrations of DA on the few occasions that it was detected. This suggests that *E. moschata* is a potential vector for DA transfer to higher trophic levels in the coastal marine food web, not excluding humans. These data, combined with known aspects of the life history of the species, are a necessary step towards achieving an understanding of the accumulation of phycotoxins in cephalopods.

Gribble, K.E., Keafer, B.A., Quilliam, M.A., Cembella, A.D., Kulis, D.M., Manahan, A., and Anderson, D.M. **Distribution and toxicity of *Alexandrium ostenfeldii* (Dinophyceae) in the Gulf of Maine, USA.** *Deep Sea Research Part II, Topical Studies in Oceanography* 52(19-21): 2745-2763, 2005.

Notes: *Alexandrium ostenfeldii* is a thecate, mixotrophic dinoflagellate recently linked to a novel suite of toxins called spirolides. This study provides the first description of the regional distribution of *A. ostenfeldii* in the Gulf of Maine (GOM), and the first report and analysis of spirolide toxicity in *A. ostenfeldii* in waters south of Nova Scotia. Morphological examination of cells in field samples and of clonal cultures isolated from several stations in the GOM confirmed the presence of *A. ostenfeldii*. A genus-specific antibody probe, and an *A. ostenfeldii* species-specific oligonucleotide probe labeled these cells; a probe specific for the North American *A. fundyense* / *tamarense* / *catenella* species complex did not label *A. ostenfeldii* cells. Cell size ranged from 20 to nearly 90 µm, and most cells contained food vacuoles, with a total vacuole size from 1 to 48 µm. The hydrographic forcings controlling the distribution of *A. ostenfeldii* in the GOM are quite similar to those acting on the *A. fundyense* population at the same time of the year. The highest concentrations of *A. ostenfeldii* were observed nearshore, to the east of Penobscot Bay, Lit times with an offshore-turning branch of high cell concentration to the south of Penobscot Bay. Casco Bay appears to be an area of accumulation for *A. ostenfeldii* cells advected toward shore from the core of the Population to the northeast. Concentrations of *A. ostenfeldii* were generally higher at the surface than deeper, except at locations where the pooling of lower-salinity water at the surface may have led to the subduction of the population flowing in from the east. PSP toxins were detected in field populations containing *A. ostenfeldii* and *A. fundyense*, but not in *A. ostenfeldii* cultures isolated from the GOM. Spirolide toxins were found in 36 of 60 field samples. More than 83% of samples containing *A. ostenfeldii* cells had one or more of spirolide congeners A, B, C2 and D2. The total concentration of spirolides per cell at each station where *A. ostenfeldii* was detected ranged from non-detectable to 282 fmol cell⁻¹. *A. ostenfeldii* cultures originating from different locations showed a great diversity in spirolide content and composition. All cultures contained spirolides, ranging from 28 to 113 fmol cell⁻¹. Spirolide congeners desMeC, C and D were present in some cultures, but were not detected in any of the field samples. Based on differences and similarities between their toxin profiles, five toxin phenotypes were identified. The highest per cell spirolide contents in the cultures were nearly two times lower than the highest levels observed in field samples. Currently there is no routine monitoring for spirolide toxins in shellfish in the region, but it may be necessary eventually to expand ongoing toxin monitoring in the GOM to include analysis for spirolides.

Edwards, M., Johns, D.G., Leterme, S.C., Svendsen, E., and Richardson, A.J. **Regional climate change and harmful algal blooms in the northeast Atlantic.** *Limnology and Oceanography* 51(2): 820-829, 2006.

Notes: We investigated long-term spatial variability in a number of Harmful Algal Blooms (HABs) in the northeast Atlantic and North Sea using data from the Continuous Plankton Recorder. Over the last four decades some dinoflagellate taxa showed pronounced variation in the south and east of the North Sea, with the most significant increases being restricted to the adjacent waters off Norway. There was also a general decrease along the eastern coast of the United Kingdom. The most prominent feature in the interannual bloom frequencies over the last four decades was the anomalously high values recorded

in the late 1980s in the northern and central North Sea areas. The only mesoscale area in the northeast Atlantic to show a significant increase in bloom formation over the last decade was the Norwegian coastal region. The changing spatial patterns of HAB taxa and the frequency of bloom formation are discussed in relation to regional climate change, in particular, changes in temperature, salinity, and the North Atlantic Oscillation (NAO). Areas highly vulnerable to the effects of regional climate change on HABs are Norwegian coastal waters and the Skagerrak. Other vulnerable areas include Danish coastal waters, and to a lesser extent, the German and Dutch Bight and the northern Irish Sea. Quite apart from eutrophication, our results give a preview of what might happen to certain HAB genera under changing climatic conditions in temperate environments and their responses to variability of climate oscillations such as the NAO.

Dittmann, E. and Wiegand, C. **Cyanobacterial toxins - occurrence, biosynthesis and impact on human affairs.** *Molecular Nutrition and Food Research* 50(1): 7-17, 2006.

Notes: Mass developments of cyanobacteria ("blue-green algae") in lakes and brackish waters have repeatedly led to serious concerns due to their frequent association with toxins. Among these are the widespread hepatotoxins microcystin (MC) and nodularin (NOD). Here, we give an overview about the ecostrategies of the diverse toxin-producing species and about the genes and enzymes that are involved in the biosynthesis of the cyclic peptides. We further summarize current knowledge about toxicological mechanisms of MC and NOD, including protein phosphatase inhibition, oxidative stress and their tumor-promoting capabilities. One biotransformation pathway for MC is described. Mechanisms of cyanobacterial neurotoxins (anatoxin-a, homanatoxin-a, and anatoxin-a(s)) are briefly explained. We highlight selected cases of human fatalities related to the toxins. A special focus is given to evident cases of contamination of food supplements with cyanobacterial toxins, and to the necessary precautions.

Garcia, R. and Johnstone, R.W. **Effects of *Lyngbya majuscula* (Cyanophyceae) blooms on sediment nutrients and meiofaunal assemblages in seagrass beds in Moreton Bay, Australia.** *Marine and Freshwater Research* 57(2): 155-165, 2006.

Notes: Blooms of *Lyngbya majuscula* have been increasingly recorded in the waters of Moreton Bay, on the south-east coast of Queensland, Australia. The influences of these blooms on sediment infauna and the implications for sediment biogeochemical processes was studied. Sediment samples were taken from Moreton Bay banks during and after the bloom season. The deposition of *L. majuscula* seems to be responsible for the higher total Kjeldahl nitrogen (TKN) concentrations measured during the bloom period. Total organic carbon (TOC) concentrations did not change. *Lyngbya majuscula* blooms had a marked influence on the meiobenthos. Nematodes, copepods and polychaetes were the most abundant groups of meiofauna, and the bloom produced a decrease in the abundance and a change in the sediment depth distribution of these organisms. The distribution of nematodes, copepods and polychaetes in sediment became shallower. Further, the bloom did not affect the abundance and distribution of polychaetes as strongly as it did copepods and nematodes. The changes observed in the distribution of meiofauna in the sediment during the bloom period indicate that *L. majuscula* produces oxygen depletion in sediments, and that different fauna seem to be affected to different degrees.

Hu, C., Muller-Karger, F.E., and Swarzenski, P.W. **Hurricanes, submarine groundwater discharge, and Florida's red tides.** *Geophysical Research Letters* 33(11): art. L11601, 2006.

Notes: A *Karenia brevis* Harmful Algal Bloom affected coastal waters shallower than 50 m off west-central Florida from January 2005 through January 2006, showing a sustained anomaly of ~1 mg chlorophyll m⁻³ over an area of up to 67,500 km². Red tides occur in the same area (approximately 26-29°N, 82-83°W) almost every year, but the intense 2005 bloom led to a widespread hypoxic zone (dissolved oxygen <2 mg L⁻¹) that caused mortalities of benthic communities, fish, turtles, birds, and marine mammals. Runoff alone provided insufficient nitrogen to support this bloom. We pose the hypothesis that submarine groundwater discharge (SGD) provides the missing nutrients, and indeed can trigger and support the recurrent red tides off west-central Florida. SGD inputs of dissolved inorganic nitrogen (DIN) in Tampa Bay alone are ~35% of that discharged by all central Florida rivers draining west combined. We propose that the unusual number of hurricanes in 2004 resulted in high runoff, and in higher than normal SGD emerging along the west Florida coast throughout 2005, initiating and fueling the persistent HAB. This mechanism may also explain recurrent red tides in other coastal regions of the Gulf of Mexico.

Mitra, A. and Flynn, K.J. **Promotion of harmful algal blooms by zooplankton predatory activity.** *Biology Letters* 2(2): 194-197, 2006.

Notes: The relationship between algae and their zooplanktonic predators typically involves consumption of nutrients by algae, grazing of the algae by zooplankton which in turn enhances predator biomass, controls algal growth and regenerates nutrients. Eutrophication raises nutrient levels, but does not simply increase normal predator-prey activity; rather, harmful algal bloom (HAB) events develop often with serious ecological and aesthetic implications. Generally, HAB species are outwardly poor competitors for nutrients, while their development of grazing deterrents during nutrient stress ostensibly occurs too late, after the nutrients have largely been consumed already by fast-growing non-HAB species. A new mechanism is presented to explain HAB dynamics under these circumstances. Using a multi-nutrient predator-prey model, it is demonstrated that these blooms can develop through the self-propagating failure of normal predator-prey activity, resulting in the transfer of nutrients into HAB growth at the expense of competing algal species. Rate limitation of this transfer provides a continual level of nutrient stress that results in HAB species exhibiting grazing deterrents protecting them from top-down control. This process is self-stabilizing as long as nutrient demand exceeds supply, maintaining the unpalatable status of HABs; such events are most likely under eutrophic conditions with skewed nutrient ratios.

Malbrouck, C. and Kestemont, P. **Effects of microcystins on fish.** *Environmental Toxicology and Chemistry* 25(1): 72-86, 2006.

Notes: Microcystins (MCs) are hepatotoxic heptapeptides released into water during or on senescence of cyanobacterial blooms. This review details the different effects of the MCs on fish and discusses their potential consequences in aquatic food webs. In early life stages, exposure to MCs causes, in a dose-dependent manner, perturbations to embryonic hatching, decrease in survival and growth rate, as well as histopathological effects (enlarged and opaque yolk sac, small head, curved body and tail, hepatobiliary abnormalities, ultrastructural alterations in hepatocytes, heart rate perturbations). In adults and juveniles, field and experimental studies demonstrated that after ingestion MCs accumulate mainly in liver but can also be found in muscle and viscera. Microcystin exposure has been shown to affect growth rate and osmoregulation, increase liver enzyme activities in the serum and heart rate, modify behavior, and exert histopathological effects in the liver, intestine, kidneys, heart, spleen, or gills, but the degree to which these effects were seen depends on the exposure route. The detoxication pathway of MCs in fish begins with a conjugation reaction to glutathione catalyzed by glutathione S-transferases, and this is comparable to the reaction demonstrated in other organisms, from plants to mammals. It appears that MC concentrations found in nature can potently affect several trophic levels in the aquatic ecosystems, in particular by inducing failure of sensitive stages (e.g., fish fry) to develop and accumulating in the food chains. The need of further quantitative studies on the sublethal effects, accumulation, and fate of MCs in aquatic food chains still remains.

Rhodes, L.L., Adamson, J.E., Rublee, P.A., and Schaefer, E. **Geographic distribution of *Pfiesteria* spp. (Pfiesteriaceae) in Tasman Bay and Canterbury, New Zealand (2002-03).** *New Zealand Journal of Marine and Freshwater Research* 40(1): 211-220, 2006.

Notes: The fish-killing heterotrophic dinoflagellate species *Pfiesteria piscicida* and *P. shumwayae* (also *Pseudopfiesteria shumwayae*) were present throughout New Zealand and were residents of Tasman Bay's well-flushed estuaries and Canterbury's brackish lakes, as determined by polymerase chain reaction-based detection assays. The two species occurred in a wide range of salinities and temperatures, although detection was restricted seasonally from spring through to autumn, except for one incidence in the shallow waters of Wairewa/Lake Forsyth, Canterbury, in the winter of 2003. The DNA sequencing data indicated that *P. shumwayae* may encompass a suite of genetically closely related species. The presence of *Pfiesteria* in New Zealand is not considered an immediate risk to fish or human health given the current low-to-moderate nutrient concentrations in New Zealand's estuaries and brackish lakes. However, increases in nutrient loadings could pose a risk, as has occurred in eastern United States estuaries.

James, K.J., Gillman, M., Amandi, M.F., López-Rivera, A., Puente, P.F., Lehane, M., Mitrovic, S., and Furey, A. **Amnesic shellfish poisoning toxins in bivalve molluscs in Ireland.** *Toxicon* 46(8): 852-858, 2005.

Notes: In December 1999, domoic acid (DA) a potent neurotoxin, responsible for the syndrome Amnesic Shellfish Poisoning (ASP) was detected for the first time in shellfish harvested in Ireland. Two liquid chromatography (LC) methods were applied to quantify DA in shellfish after sample clean-up using solid-phase extraction (SPE) with strong anion exchange (SAX) cartridges. Toxin detection was achieved using photodiode array ultraviolet (LC-UV) and multiple tandem mass spectrometry (LC-MSⁿ). DA was identified in four species of bivalve shellfish collected along the west and south coastal regions of the Republic of Ireland. The amount of DA that was present in three species was within EU guideline limits for sale of shellfish (20µgDA/g); mussels (*Mytilus edulis*), <1.0µgDA/g; oysters (*Crassostrea edulis*), <5.0µgDA/g and razor clams (*Ensis siliqua*), <0.3µgDA/g. However, king scallops (*Pecten maximus*) posed a significant human health hazard with levels up to 240µgDA/g total tissues. Most scallop samples (55%) contained DA at levels greater than the regulatory limit. The DA levels in the digestive glands of some samples of scallops were among the highest that have ever been recorded (2820µgDA/g).

Leverone, J.R., Blake, N.J., Pierce, R.H., and Shumway, S.E. **Effects of the dinoflagellate *Karenia brevis* on larval development in three species of bivalve mollusc from Florida.** *Toxicon* 48(1): 75-84, 2006.

Notes: The effects of *Karenia brevis* (Wilson clone) on larval survival and development of the northern quahog, *Mercuraria mercenaria*, eastern oyster, *Crassostrea virginica* and bay scallop, *Argopecten irradians*, were studied in the laboratory. Larvae were exposed to cultures of whole and lysed cells, with mean total brevetoxin concentrations of 53.8 and 68.9µg/L¹, respectively. Survival of early (3-day-old) larvae was generally over 85% for all shellfish species at *K. brevis* densities of 100 cells/ml¹ or less, and not significantly different between whole and lysed culture. At 1000 cells/ml¹, survival was significantly less in lysed culture than whole culture for both *M. mercenaria* and *C. virginica*. Survival of late (7-day-old) larvae in all three species was not significantly affected by *K. brevis* densities of 1000 cells/ml¹ or less. At 5000 cells/ml¹, however, survival was reduced to 37%, 26% and 19% for *A. irradians*, *M. mercenaria* and *C. virginica*, respectively. Development of *C. virginica* and *M. mercenaria* larvae was protracted at *K. brevis* densities of 1000 cells/ml¹. These results suggest that blooms of *K. brevis*, and particularly their associated brevetoxins, may have detrimental consequences for Florida's shellfisheries by disrupting critical larval processes. Special attention should be paid to blooms of *K. brevis* where these shellfish occur naturally or where aquaculture and restoration activities are either ongoing or planned.

Bowden, B.F. **Yessotoxins -- polycyclic ethers from dinoflagellates: Relationships to diarrhetic shellfish toxins.** *Toxin Reviews* 25(2): 137-157, 2006.

Notes: Diarrhetic shellfish poisoning (DSP) is known to be caused by okadaic acid (OA), dinophysistoxin-1 (DTX-1), and pectenotoxins (PTX) that are polycyclic ethers produced by marine dinoflagellates, mainly *Dinophysis* sp. Yessotoxin (YTX) is also classified as a DSP toxin, because it was first isolated in 1987 from scallops that were implicated in a DSP episode. Twenty two structural analogs of yessotoxin have now been isolated either from shellfish, or the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum*. Metabolism of YTX to 45-hydroxyYTX, carboxyYTX, and hydroxycarboxyYTX in mussels has been reported. However, most yessotoxin analogs initially isolated from shellfish extractions now also have been isolated from cultures of *P. reticulatum*. The pharmacological activity of the yessotoxin group is distinctly different from those of OA, DTX-1, and PTX and this has fueled a move to remove YTX from the DSP group. This review focuses on advances in the chemistry, pharmacology, and detection of yessotoxins.

Llewellyn, L., Negri, A., and Robertson, A. **Paralytic shellfish toxins in tropical oceans.** *Toxin Reviews* 25(2): 159-196, 2006.

Notes: The tropics possess some of the world's richest marine environments, most notably coral reefs. Concealed within these ecosystems are a group of potent neurotoxins called the paralytic shellfish toxins (PSTs), the most famous of which is saxitoxin. Thirty years ago, PSTs were recognized as a major danger to seafood consumers in the tropics. The tropical dinoflagellate *Pyrodinium bahamense* biosynthesizes PSTs and its contamination of seafood has caused more illnesses and deaths than any other PST-producing microalga. Apart from this and other dinoflagellates, PSTs have been confirmed in tropical

benthic algae, molluscs, echinoderms, crustacea, and other arthropods. Some of these organisms are unique in that, to date, they have only been found to be toxic in tropical oceans. For example, species of grazing and predatory gastropods, crabs, and more recently cephalopods have been discovered to contain PSTs in a number of intertidal tropical locations. These animals are thought to accumulate the toxins from benthic sources rather than toxic dinoflagellates as happens with filter-feeding bivalve molluscs such as clams and oysters. Here we evaluate the current understanding of PST transmission through tropical food webs. Finally, we consider the prevalence of PST intoxications in tropical regions and their social and economic costs.

Lapointe, B.E., Barile, P.J., Littler, M.M., Littler, D.S., Bedford, B.J., and Gasque, C. **Macroalgal blooms on southeast Florida coral reefs I. Nutrient stoichiometry of the invasive green alga *Codium isthmocladum* in the wider Caribbean indicates nutrient enrichment.** *Harmful Algae* 4(6): 1092-1105, 2005.

Notes: Invasive blooms of the siphonaceous green algae *Codium* spp. have been considered a symptom of coastal eutrophication but, to date, only limited biochemical evidence supports a linkage to land-based nutrient pollution. Beginning in the summer of 1990, spectacular blooms of unattached *Codium isthmocladum* developed on deep coral reef habitats in southern Palm Beach County and northern Broward County, and in subsequent years, attached populations formed on reefs in northern Palm Beach County. To better understand the nutrition of these HABs, we collected *C. isthmocladum* and other reef macroalgae from various locations in southeast Florida as well as the wider Caribbean region for tissue C:N:P analysis in order to gauge variability in the type and degree of N- and/or P-limited growth. Widespread nutrient enrichment in Floridian *C. isthmocladum* populations was evidenced by significantly higher tissue P (0.06% versus 0.04% of dry weight) and lower C:N (12 versus 19), C:P (425 versus 980), and N:P (35 versus 50) ratios compared to more nutrient-depleted Caribbean populations. To determine nutrient availability on southeast Florida's reefs, we sampled near-bottom waters at a variety of locations for DIN (NH₄⁺+NO₃⁻+NO₂⁻) and SRP analysis. In general, concentrations of NH₄⁺, NO₃⁻ and SRP were all high on southeast Florida's reefs compared to values reported for Caribbean coral reefs. Although summertime upwelling provides episodic NO₃⁻ and SRP enrichment to reefs in southeast Florida, these transient nutrient pulses have not historically supported *C. isthmocladum* blooms. We suggest that the widespread P enrichment of *C. isthmocladum* tissue and water column DIN:SRP ratios <16:1 in southeast Florida drive this system toward N limitation where low level NH₄⁺ enrichment becomes of paramount importance. Hence, the recent *C. isthmocladum* blooms appear to be supported by increasing land-based nutrient pollution, particularly, sewage that is enriched in NH₄⁺ and SRP at a low N:P ratio (<10:1) critical to sustaining balanced growth during bloom formation.

Lapointe, B.E., Barile, P.J., Littler, M.M., and Littler, D.S. **Macroalgal blooms on southeast Florida coral reefs II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of sewage nitrogen.** *Harmful Algae* 4(6): 1106-1122, 2005.

Notes: Since 1990, coral reefs off southeast Florida have experienced an unprecedented succession of macroalgal blooms and invasions. To determine if anthropogenic land-based nitrogen (N) sources support these HABs, we collected macroalgal tissue for stable nitrogen isotope ($\delta^{15}\text{N}$) analysis at three spatially distinct depths ranging from the shallow subtidal to the shelf break (43m) along seven transects from Jupiter to Deerfield Beach, Florida, USA. This sampling was initiated during a historically significant drought in the spring of 2001 when rainfall, stormwater runoff, and upwelling were at a minimum. The sampling was repeated in the summer wet season following significant stormwater runoff and during a strong upwelling event. Despite significant seasonal changes in N source availability, $\delta^{15}\text{N}$ values did not vary between samplings. Collectively, $\delta^{15}\text{N}$ values were significantly higher on inshore shallow reefs (+8.1‰) compared to mid (+6.1‰) and deep reefs (+6.7‰). Values were also elevated in the southern portion of the study area (e.g., Boca Raton, +8.5‰) where nearly 1.5 billion l/day of secondarily treated wastewater is discharged into the ocean via coastal outfalls. *Codium isthmocladum*, a macroalga that assimilates its nutrients entirely from the water column, was the dominant bloom species in the southern study area, where tissue $\delta^{15}\text{N}$ values matched source values of nearby sewage outfalls. The northern study area was dominated by species of the genus *Caulerpa*, particularly the invasive Pacific native *C. brachypus* var. *parvifolia*, which are capable of accessing benthic nutrient sources (e.g., submarine groundwater discharge, SGD) by means of root-like rhizoids. The northern area does not have sewage outfalls but features a highly transmissive geologic zone where SGD may be enriched with septic tank leachate and effluent from municipal deep injection wells. Multiple lines of evidence supported the hypothesis that land-based sewage N was more important than upwelling as a N source to these HABs: (1) $\delta^{15}\text{N}$ values were highest on shallow reefs and decreased with increasing depth, indicating land-based sources of enrichment; (2) elevated $\delta^{15}\text{N}$ values occurred in these HABs during the dry season, prior to the onset of the summer upwelling; (3) elevated NH₄⁺ concentrations occur on these reefs during both

upwelling and non-upwelling periods and are kinetically preferred by macroalgae compared to upwelled NO₃⁻. These findings provide a case study of a coupling between increasing anthropogenic activities and the development of macroalgal HABs, including invasive species that threaten economically important reef resources in southeast Florida.

Seaborn, D.W., Tengs, T., Cerbin, S., Kokocinski, M., and Marshall, H.G. **A group of dinoflagellates similar to *Pfiesteria* as defined by morphology and genetic analysis.** *Harmful Algae* 5(1): 1-8, 2006.

Notes: Eight novel *pfiesteria*-similar dinoflagellates are described that form a genetically well-defined group with distinct morphological features, and are capable of feeding myzocytotically on cryptophytes and other algae. Taxonomically, there was concordance between the genetic analysis and plate tabulation features among these taxa. It is suggested that these dinoflagellates are important grazers of other phytoplankton in the environment, and their total ecological significance has been underestimated.

Persich, G.R., Kulis, D.M., Lilly, E.L., Anderson, D. M., and Garcia, V. M. T. **Probable origin and toxin profile of *Alexandrium tamarense* (Lebour) Balech from southern Brazil.** *Harmful Algae* 5(1): 36-44, 2006.

Notes: The distribution of the toxic dinoflagellate *Alexandrium tamarense* Lebour has apparently expanded within the southern hemisphere during the last 2 decades. Toxic blooms of *A. tamarense* were recorded in Argentinean coastal waters since 1980; however, the first documented bloom in southern Brazil was in 1996. In this study, 13 strains of *A. tamarense* from southern Brazil were isolated and kept in culture. Phylogenetic analysis using RFLP and DNA sequences of the D1-D2 region of large subunit ribosomal DNA (rDNA) clearly indicates that Brazilian strains are most closely related to other South American strains. The strains from South America are placed firmly within a phylogenetic clade which contains strains from North America, northern Europe and northern Asia, previously called the North American clade. Possible dispersal hypotheses are discussed. The cultures were also analyzed for saxitoxin and its derivatives by high performance liquid chromatography (HPLC). The main saxitoxin groups found were the low toxicity N-sulfocarbamoyl group, Cl, 2 (30-84%), followed by the high potency carbamate toxins, gonyautoxins 1, 4 (6.6-55%), gonyautoxins 2, 3 (0.3-29%), neosaxitoxin (1.4-24%) and saxitoxin (0-4.4%). The toxin composition is similar to that of other strains from South America, supporting a close relationship between *A. tamarense* from southern Brazil and other areas of South America. Toxicity values were variable (7.07-65.92 pg STX cell⁻¹), with the higher range falling among the most toxic values recorded for cultures of *A. tamarense*, indicating the significant risk for shellfish contamination and human intoxication during blooms of this species along the southern Brazilian coast.

Busse, L.B., Venrick, E.L., Antrobus, R., Miller, P.E., Vigilant, V., Silver, M.W., Mengelt, C., Mydlarz, L., and Prezelin, B.B. **Domoic acid in phytoplankton and fish in San Diego, CA, USA.** *Harmful Algae* 5(1): 91-101, 2006.

Notes: We provide the first confirmation of the presence of domoic acid (DA) in phytoplankton and fish in San Diego, California, based on samples collected between 1 October 2003 and 29 September 2004. In February 2004, we detected DA in seawater samples collected off the Scripps Pier and also in coastal samples as far as 120 km to the north. At the same time we observed populations of toxic *Pseudo-nitzschia australis* and *Pseudo-nitzschia multiseries* as high as 7.7 x 10⁴ cells l⁻¹. Elevated concentrations of DA and abundances of the toxic species were also found further north in coastal waters of Orange County and, to a lesser extent, in southern Los Angeles County. DA concentrations in the viscera from four species of fish obtained at or near the Scripps Pier ranged from low to above the critical level for public safety. Samples of mussel tissues from the Scripps Pier analyzed by the State Department of Health Services contained low but detectable amounts of DA. Concomitant sea lion strandings from San Diego to Malibu Beach may be related to the presence of DA. DA in tissue from mussels and fish provides evidence for the local transfer of DA from an algal source to higher trophic levels in San Diego coastal waters.

Madigan, T.L., Lee, K.G., Padula, D.J., McNabb, P., and Pointon, A.M. **Diarrhetic shellfish poisoning (DSP) toxins in South Australian shellfish.** *Harmful Algae* 5(2): 119-123, 2006.

Notes: In December 2003 a bloom of the toxic dinoflagellate *Dinophysis acuminata* (Claparede and Lachmann, 1859) was detected during routine plankton monitoring at Smoky Bay, on the west coast of South Australia (SA). Samples of scallops, razorfish and oysters were collected and analysed by LC-MS/MS to compare diarrhetic shellfish poisoning (DSP) toxin concentrations within the different species of shellfish that were exposed to the same bloom event at the same location. Toxin concentrations were compared in shellfish samples, which were prepared in ways reflecting how the organisms may be consumed. Toxin concentrations varied between species and only the oyster sample was found to be above the Australian regulatory limit of 0.2 mg/kg (okadaic acid equivalent). Toxin concentrations in both king scallops and razor-fish were lower in samples where the viscera had been removed than in whole organism samples. The results reported here, although based on limited sampling, demonstrate for the first time the existence of a risk to public health from DSP in cultivated and natural shellfish in SA.

O'Halloran, C., Silver, M.W., Holman, T.R., and Scholin, C.A. *Heterosigma akashiwo* in central California waters. *Harmful Algae* 5(2): 124-132, 2006.

Notes: *Heterosigma akashiwo* (Hada) gives rise to red tides along the Atlantic and Pacific coasts and is known to produce brevetoxins. This investigation establishes baseline information showing the presence of *H. akashiwo* along the central California coast based on water samples collected from the Santa Cruz pier in Monterey Bay (on the open coast) and the Berkeley pier in San Francisco Bay. Light and electron microscopy as well as two species-specific DNA probe methods based on cell homogenates preparations were employed to detect *H. akashiwo* during the 2001-2002 field study. The DNA probe methods consisted of a sandwich hybridization assay (SHA), which targets ribosomal RNA (rRNA), and an end-point polymerase chain reaction (PCR) assay, which targets internal transcribed spacer (ITS) sequences of rRNA genes. The SRA was used to provide semi-quantitative data showing the intermittent presence of the species during a 13-month period in Monterey Bay. Samples that showed a variety of responses in the SHA (negative as well as the highest) were then subjected to the PCR assay in an attempt to confirm species identification using an independent DNA probe method that employs cell homogenates; samples included those from Monterey Bay and one from a red tide event in San Francisco Bay. SHA and PCR assays agreed on the presence or absence of *H. akashiwo*. Gene products from two field samples positive for *H. akashiwo* by PCR were cloned and sequenced and found to be identical to those of that species in GenBank. When the same samples were viewed by light microscopy, however, *H. akashiwo* cells were only seen in the sample with the highest abundance of that species, as evidenced by SHA. It was extremely difficult to recognize naturally occurring *H. akashiwo* using light microscopy in field samples that had been preserved with Lugol's iodine, including samples that gave positive results by cell homogenate methods. Results of this study indicate that *H. akashiwo* is present along the open California coast and could easily be missed in routine phytoplankton surveys. Despite its presence, *H. akashiwo* does not appear to routinely bloom with sufficient densities to cause harmful outbreaks of the frequency and severity documented in some other coastal environments. Molecular identification techniques may be the preferred approach over light microscopy when there is a need to rapidly screen many samples for fragile, harmful species and those that are otherwise problematic to identify based on their gross morphology alone.

Rhodes, L., McNabb, P., de Salas, M., Briggs, L., Beuzenberg, V., and Gladstone, M. **Yessotoxin production by *Gonyaulax spinifera***. *Harmful Algae* 5(2): 148-155, 2006.

Notes: Yessotoxin (YTX) contamination of greenshell (TM) mussels (*Perna canaliculus*) in the Marlborough Sounds, New Zealand, occurred in May 2004 with no known YTX-producing algae present in associated seawater samples. *Gonyaulax spinifera*, previously considered a non-YTX producer, was isolated from the seawater samples and production of YTX by the cells grown in culture was determined by identification of YTXs by ELISA. The algal identification was based on the type description and cyst form. DNA sequence data for *G. spinifera* differed from the morphologically similar *G. membranacea*, *G. digitale*, and *G. cf. spinifera*. *Protoceratium reticulatum* was isolated from the same site as *G. spinifera* 3 weeks later, but was not a YTX-producer by LC-MS and YTX ELISA analyses. Implications of the discovery that YTX is produced by *G. spinifera* are that this micro-alga will need to be included in future risk assessments for YTX based on the phytoplankton composition.

Ahn, Y.H., Shanmugam, P., Ryu, J.H., and Jeong, J.C. **Satellite detection of harmful algal bloom occurrences in Korean waters.** *Harmful Algae* 5(2): 213-231, 2006.

Notes: *Cochlodinium polykrikoides* (p) is a planktonic dinoflagellate known to produce red tides responsible for massive fish kills and thereby serious economic loss in Korean coastal waters, particularly during summer and fall seasons. The present study involved analyzing chlorophyll-a (Chl-a) from SeaWiFS ocean color imagery collected over the period 1998-2002 to understand the spatial and temporal aspects of *C. polykrikoides* blooms that occurred in the enclosed and semi-enclosed bays of the Korean Southeast Sea. NOAA-AVHRR data were used to derive Sea Surface Temperature (SST) to elucidate physical factors affecting the spatial distribution and abundance of *C. polykrikoides* blooms. The time series of SeaWiFS-derived Chl-a gave an impression that recent red tide events with higher concentrations appeared to span more than 8 weeks during summer and fall seasons and were widespread in most of the Korean Southeast Sea coastal bays and neighboring oceanic waters. Coupled eutrophication and certain oceanic processes were thought to give rise to the formation of massive *C. polykrikoides* blooms with cell abundances ranging from 1000 to 30,000 cells ml⁻¹, causing heavy mortalities of aquaculture fish and other marine organisms in these areas. Our analysis indicated that Chl-a estimates from SeaWiFS ocean color imagery appeared to be useful in demarcating the locality, spatial extent and distribution of these blooms, but unique identification of *C. polykrikoides* from non-bloom and sediment dominated waters remains unsuccessful with this data alone. Thus, the classical spectral enhancement and classification techniques such as Forward Principal Component Analysis (FPCA) and Minimum Spectral Distance (MSD) to uniquely identify and better understand *C. polykrikoides* blooms characteristics from other optical water types were attempted on both low spatial resolution SeaWiFS ocean color imagery and high spatial resolution Landsat-7 ETM+ imagery. Application of these techniques could capture intricate and striking patterns of *C. polykrikoides* blooms from surrounding non-bloom and sediment dominated waters, providing improved capability of detecting, predicting and monitoring *C. polykrikoides* bloom in such optically complex waters. The result obtained from MSD classification showed that retrieval of *C. polykrikoides* bloom from the mixed phase of this bloom with turbid waters was not feasible with the SeaWiFS ocean color imagery, but feasible with Landsat-7 ETM+ imagery that provided more accurate and comparable spatial *C. polykrikoides* patterns consistent with in situ observations. The dense phase of the bloom estimated from these imageries occupied an area of more than 25 km² around the coastal bays and the mixed phase extended over several hundreds kilometers towards the Southeast Sea offshore due to exchange of water masses caused by coastal and oceanic processes. Sea Surface temperature analyzed from AVHRR infrared data captured the northeastward flow of Tsushima Warm Current (TWC) waters that provided favorable environmental conditions for the rapid growth and subsequent southward initiation of *C. polykrikoides* blooms in hydrodynamically active regions in the Korean Southeast Sea offshore.

Arthur, K.E., Limpus, C.J., Roelfsema, C.M., Udy, J.W., and Shaw, G.R. **A bloom of *Lyngbya majuscula* in Shoalwater Bay, Queensland, Australia: An important feeding ground for the green turtle (*Chelonia mydas*).** *Harmful Algae* 5(3): 251-265, 2006.

Notes: *Lyngbya majuscula*, a toxic cyanobacterium, was observed blooming during June-July (winter) 2002 in Shoalwater Bay, Queensland, Australia, an important feeding area for a large population of green turtles (*Chelonia mydas*). The bloom was mapped and extensive mats of *L. majuscula* were observed overgrowing seagrass beds along at least 18 km of coast, and covering a surface area of more than 11 km². Higher than average rainfall preceded the bloom and high water temperatures in the preceding summer may have contributed to the bloom. In bloom samples, lyngbyatoxin A (LA) was found to be present in low concentration (26 mg kg⁻¹ (dry weight)), but debromoaplysiatoxin (DAT) was not detected. The diet of 46 green turtles was assessed during the bloom and *L. majuscula* was found in 51% of the samples, however, overall it contributed only 2% of the animals' diets. *L. majuscula* contribution to turtle diet was found to increase as the availability of the cyanobacterium increased. The bloom appeared to have no immediate impact on turtle body condition, however, the presence of a greater proportion of damaged seagrass leaves in diet in conjunction with decreases in plasma concentrations of sodium and glucose could suggest that the turtles may have been exposed to a substandard diet as a result of the bloom. This is the first confirmed report of *L. majuscula* blooming in winter in Shoalwater Bay, Queensland, Australia and demonstrates that turtles consume the toxic cyanobacterium in the wild, and that they are potentially exposed to tumour promoting compounds produced by this organism.

Hsia, M.H., Morton, S.L., Smith, L.L., Beauchesne, K.R., Huncik, K.M., and Moeller, P.D.R. **Production of goniodomin A by the planktonic, chain-forming dinoflagellate *Alexandrium monilatum* (Howell) Balech isolated from the Gulf**

Notes: The chain-forming, dinoflagellate *Alexandrium monilatum* has been reported to be associated with widespread discolored water and increased fish mortality in the Mississippi Sound and off the eastern and western coasts of Florida. Previous studies over the last 60-70 years have determined that *A. monilatum* produces a harmful substance(s) that is predominantly contained in the cell mass as exhibited by apparent increased toxicity when the organism cytolyses. The current research in our lab corroborated earlier research demonstrating that *A. monilatum* produces a lipophilic toxin, unlike its *Alexandrium* relatives noted for their production of saxitoxin-like toxins. Using sophisticated chemical, chromatographic, and analytical techniques, we have successfully purified and identified the molecular structure of the toxin produced by *A. monilatum*. We utilized a 500 MHz NMR to carry out a number of experiments (i.e., H-1, C-13, COSY, HSQC, and HMBC) to unambiguously determine the molecular structure of the toxin. In addition, we report mass analysis of the toxin utilizing electrospray ionization-mass spectrometry (ESI-MS), matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS), and Q-TOF mass spectral techniques. The toxin is representative of a polyether macrolide with an empirical formula of C₄₃H₆₀O₁₂. This toxic compound is shown to be identical to a Japanese tidepool toxin identified as goniodomin A, which is produced by another *Alexandrium* species.
