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Marine Science Review – 405

Blooms and biotoxins

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In this review:

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A. Recent articles with abstracts

Dorantes-Aranda, J.J., García-de la Parra, L.M., Alonso-Rodríguez, R., Morquecho, L., and Voltolina, D. Toxic effect of the harmful dinoflagellate *Cochlodinium polykrikoides* on the spotted rose snapper *Lutjanus guttatus*. *Environmental Toxicology* 25(4): 319-326, 2010.

Notes: The dinoflagellate *Cochlodinium polykrikoides* isolated from Bahía de La Paz, Gulf of California, showed an important short-term toxic effect on the spotted rose snapper *Lutjanus guttatus*. This microalga was able to decrease fish liver catalase activity and lipid peroxidation. Fish exposed to live dinoflagellates developed an abnormal mucus secretion on the gills that was directly related to algal cell concentration. Hepatic catalase inhibition and an increase in mucus secretion on the gills occurred when fish were exposed to 2×10^6 cells L^{-1} of *C. polykrikoides*. Lipid peroxidation was significantly different at 4×10^6 cells L^{-1} and the hepatosomatic index decreased at 3×10^6 cells L^{-1}. Our results suggest that oxidative stress contributes, at least in part, to the ichthyotoxic effect of *C. polykrikoides* from the Gulf of California.

Basti, L. and Segawa, S. Mortality of the short-neck clam *Ruditapes philippinarum* induced by the toxic dinoflagellate *Heterocapsa circularisquama*. *Fisheries Science* 76(4): 625-631, 2010.

Notes: Mortality of the short-neck clam *Ruditapes philippinarum* exposed to the toxic dinoflagellate *Heterocapsa circularisquama* was studied under controlled conditions to clarify the mechanisms of recurrent mass deaths of clams occurring in western Japanese coastal areas. One-week mortality tests, involving three water temperatures, six *H. circularisquama* concentrations, and two clam body sizes, showed a significant increase in mortality with increasing temperature, *H. circularisquama* concentration, exposure duration, and body size (ANOVA, $P < 0.01$). Clam death was observed at concentrations as low as 50 cells/ml and temperatures as low as 15 ºC. Prior to death, clams showed an extreme retraction of their mantle edge and siphon, along with recurrent vomiting behavior before initiating a closure reaction followed by paralysis then death. Gills of paralyzed clams showed an important uptake of dye, implying gill damage. This study is the first laboratory evidence of bivalve mortality induced by *H. circularisquama* at low concentrations and low temperature, and the first report of differential effects according to the body size of bivalves.

Flewelling, L.J., Adams, D.H., Naar, J.P., Atwood, K.E., Granholm, A.A., O'Dea, S.N., and Landsberg, J.H. Brevetoxins in sharks and rays (Chondrichthyes, Elasmobranchii) from Florida coastal waters. *Marine Biology* 157(9): 1937-1953, 2010.

Notes: In October 2000, a mass mortality of blacktip sharks (*Carcharhinus limbatus*) and Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) in northwest Florida occurred in conjunction with a *Karenia brevis* red tide bloom. Before this incident, no information existed on red tide-induced shark mortalities or baseline brevetoxin levels in sharks and rays from red tide-endemic areas. We report here that brevetoxin accumulation in live and red tide-killed elasmobranchs is common during

K. brevis blooms and non-bloom periods. Strong relationships were found between the frequency of red tide blooms and the average brevetoxin concentrations in elasmobranch tissues. The presence of brevetoxins in Atlantic coast sharks in the absence of documented *K. brevis* blooms may suggest that blooms are occurring in areas that are not well monitored. Although red tide-related shark mortalities are rarely observed, the presence of brevetoxins in shark embryos raises questions about the effects these toxins may have on the reproductive success of sharks.

Touzet, N., Davidson, K., Pete, R., Flanagan, K., McCoy, G.R., Amzil, Z., Maher, M., Chapelle, A., and Raine, R. Co-occurrence of the West European (Gr.III) and North American (Gr.I) ribotypes of *Alexandrium tamarense* (Dinophyceae) in Shetland, Scotland. *Protist* 161(3): 370-384, 2010.

Notes: An investigation into the diversity of the dinoflagellate *Alexandrium* was carried out during August 2007 within two fjordic sea lochs in the Shetland Isles, Scotland. The co-occurrence in the water column of the non-toxic West European (W.E. or Gr.III) and the neurotoxic North American (N.A. or Gr.I) ribotypes of *A. tamarense* was demonstrated using fluorescent in situ hybridisation. A patch of *A. tamarense* (W.E.) localised at similar to 10 m depth and extending over 6 km was detected in 'Clift Sound' with concentrations locally reaching ~1 x 10^4 cells l^{-1}. *A. tamarense* (N.A.) was also observed there but despite the presence of toxins in net haul samples collected locally, concentrations were low and near limits of detection. *Alexandrium* concentrations were ~1.5 x 10^3 cells l^{-1} in 'Vaila Sound', where both W.E. and N.A. ribotypes were detected with equal relative abundances in some samples. Given the patchiness of *A. tamarense* populations and their possible organisation in thin layer structures, better vertical resolution through fine-scale sampling will be necessary for population dynamic studies. Implications for the shellfish industry are substantial since harmful microalgae patches may not be detected during routine monitoring. Moreover, the co-occurrence of morphologically indistinct toxic and non-toxic ribotypes will necessitate implementing molecular methods for their discrimination.

Mazzillo, F.F.M., Pomeroy, C., Kuo, J., Ramondi, P.T., Prado, R., and Silver, M.W. Angler exposure to domoic acid via consumption of contaminated fishes. *Aquatic Biology* 9(1): 1-12, 2010. O/A

Notes: Domoic acid (DA) is a neurotoxin that causes amnesic shellfish poisoning, and fish are recognized vectors of DA to marine fauna. However, the exposure of anglers through consumption of DA-contaminated fish is unknown. We measured DA in 11 fish species targeted by Santa Cruz Wharf (SCW) anglers in Monterey Bay, California, USA, and surveyed anglers regarding their fish consumption patterns. In addition, we used California mussel *Mytilus californianus* DA data provided by the state of California and our measurements of DA in seawater to examine the associations between DA in fish viscera versus in mussels and seawater. DA was detected in the viscera of 7 fish species commonly consumed by anglers, and toxin uptake in fishes varied according to their diet. DA was almost entirely in the viscera, with low DA concentrations detected in muscle tissue. The majority of anglers (58% of 565) reported consuming their catch, with a small fraction ingesting the viscera. Total DA concentrations in fish decreased significantly after 11 mo storage at -20 ºC. DA concentration in seawater and California mussels was correlated with DA in the viscera of some but not all fish groups. We conclude that SCW anglers who consume their catch are exposed to asymptomatic DA doses, and that exposure is a function of the species and parts consumed, as well as storage methods and DA levels in the seawater when the fish are caught.

Choi, H., Engene, N., Smith, J.E., Preskitt, L.B., and Gerwick, W.H. Crossbyanols A–D, toxic brominated polyphenyl ethers from the Hawai'ian bloom-forming cyanobacterium *Leptolyngbya crossbyana*. *Journal of Natural Products* 73(4): 517-522, 2010.

Notes: Periodically, the marine cyanobacterium *Leptolyngbya crossbyana* forms extensive blooms on Hawai'ian coral reefs and results in significant damage to the subtending corals. Additionally, corals near mats of this cyanobacterium, but not directly overgrown, have been observed to undergo bleaching. Therefore, samples of this cyanobacterium were chemically investigated for bioactive secondary metabolites that might underlie this toxicity phenomenon. ^{1}H NMR spectroscopy-guided fractionation led to the isolation of four heptabrominated polyphenolic ethers, crossbyanols A–D (1–4). Structure elucidation of these

compounds was made challenging by their very low proton to carbon (H/C) ratio, but was completed by combining standard NMR and MS data with 2 Hz-optimized HMBC data. Derivatization of crossbyanol A as the diacetate assisted in the assignment of its structure. Crossbyanol B (2) showed antibiotic activity with an MIC value of 2.0-3.9 μg/mL against methicillin-resistant *Staphylococcus aureus* (MRSA) and relatively potent brine shrimp toxicity (IC_{50} 2.8 ppm).

Costa, L.G., Giordano, G., and Faustman, E.M. Domoic acid as a developmental neurotoxin. *NeuroToxicology* 31(5): 409-423, 2010.

Notes: Domoic acid (DomA) is an excitatory amino acid which can accumulate in shellfish and finfish under certain environmental conditions. DomA is a potent neurotoxin. In humans and in non-human primates, oral exposure to a few mg/kg DomA elicits gastrointestinal effects, while slightly higher doses cause neurological symptoms, seizures, memory impairment, and limbic system degeneration. In rodents, which appear to be less sensitive than humans or non-human primates, oral doses cause behavioral abnormalities (e.g. hindlimb scratching), followed by seizures and hippocampal degeneration. Similar effects are also seen in other species (from sea lions to zebrafish), indicating that DomA exerts similar neurotoxic effects across species. The neurotoxicity of DomA is ascribed to its ability to interact and activate the AMPA/KA receptors, a subfamily of receptors for the neuroexcitatory neurotransmitter glutamate. Studies exploring the neurotoxic effects of DomA on the developing nervous system indicate that DomA elicits similar behavioral, biochemical and morphological effects as in adult animals. However, most importantly, developmental neurotoxicity is seen at doses of DomA that are one to two orders of magnitude lower than those exerting neurotoxicity in adults. This difference may be due to toxicokinetic and/or toxicodynamic differences. Estimated safe doses may be exceeded in adults by high consumption of shellfish contaminated with DomA at the current limit of 20 μg/g. Given the potential higher susceptibility of the young to DomA neurotoxicity, additional studies investigating exposure to, and effects of this neurotoxin during brain development are warranted.

Miller, M.A. *et al.* Evidence for a novel marine harmful algal bloom: Cyanotoxin (microcystin) transfer from land to sea otters. *PLoS ONE* 5(9): art. e12576, 2010. O/A

Notes: "Super-blooms" of cyanobacteria that produce potent and environmentally persistent biotoxins (microcystins) are an emerging global health issue in freshwater habitats. Monitoring of the marine environment for secondary impacts has been minimal, although microcystin-contaminated freshwater is known to be entering marine ecosystems. Here we confirm deaths of marine mammals from microcystin intoxication and provide evidence implicating land-sea flow with trophic transfer through marine invertebrates as the most likely route of exposure. This hypothesis was evaluated through environmental detection of potential freshwater and marine microcystin sources, sea otter necropsy with biochemical analysis of tissues and evaluation of bioaccumulation of freshwater microcystins by marine invertebrates. Ocean discharge of freshwater microcystins was confirmed for three nutrient-impaired rivers flowing into the Monterey Bay National Marine Sanctuary, and microcystin concentrations up to 2,900 ppm (2.9 million ppb) were detected in a freshwater lake and downstream tributaries to within 1 km of the ocean. Deaths of 21 southern sea otters, a federally listed threatened species, were linked to microcystin intoxication. Finally, farmed and free-living marine clams, mussels and oysters of species that are often consumed by sea otters and humans exhibited significant biomagnification (to 107 times ambient water levels) and slow depuration of freshwater cyanotoxins, suggesting a potentially serious environmental and public health threat that extends from the lowest trophic levels of nutrient-impaired freshwater habitat to apex marine predators. Microcystin-poisoned sea otters were commonly recovered near river mouths and harbors and contaminated marine bivalves were implicated as the most likely source of this potent hepatotoxin for wild otters. This is the first report of deaths of marine mammals due to cyanotoxins and confirms the existence of a novel class of marine "harmful algal bloom" in the Pacific coastal environment; that of hepatotoxic shellfish poisoning (HSP), suggesting that animals and humans are at risk from microcystin poisoning when consuming shellfish harvested at the land-sea interface.

Schwacke, L.H., Twiner, M.J., DeGuise, S., Balmer, B.C., Wells, R.S., Townsend, F.I., Rotstein, D.C., Varela, R.A., Hansen, L.J., Zolman, E.S., Spradlin, T.R., Levin, M., Leibrecht, H., Wang, Z. H., and Rowles, T.K. Eosinophilia and biotoxin exposure in bottlenose dolphins (*Tursiops truncatus*) from a coastal area impacted by repeated mortality events. *Environmental Research* 110(6): 548-555, 2010.

Notes: Bottlenose dolphins (*Tursiops truncatus*) inhabiting coastal waters in the northern Gulf of Mexico have been impacted by recurrent unusual mortality events over the past few decades. Several of these mortality events along the Florida panhandle have been tentatively attributed to poisoning from brevetoxin produced by the dinoflagellate *Karenia brevis*. While dolphins in other regions of the Florida coast are often exposed to *K. brevis* blooms, large-scale dolphin mortality events are relatively rare and the frequency and magnitude of die-offs along the Panhandle raise concern for the apparent vulnerability of dolphins in this region. We report results from dolphin health assessments conducted near St. Joseph Bay, Florida, an area impacted by 3 unusual die-offs within a 7-year time span. An eosinophilia syndrome, manifested as an elevated blood eosinophil count without obvious cause, was observed in 23% of sampled dolphins. Elevated eosinophil counts were associated with decreased T-lymphocyte proliferation and increased neutrophil phagocytosis. In addition, indication of chronic low-level exposure to another algal toxin, domoic acid produced by the diatom *Pseudo-nitzschia* spp., was determined. Previous studies of other marine mammal populations exposed recurrently to *Pseudo-nitzschia* blooms have suggested a possible link between the eosinophilia and domoic acid exposure. While the chronic eosinophilia syndrome could over the long-term produce organ damage and alter immunological status and thereby increase vulnerability to other challenges, the significance of the high prevalence of the syndrome to the observed mortality events in the St. Joseph Bay area is unclear. Nonetheless, the unusual immunological findings and concurrent evidence of domoic acid exposure in this sentinel marine species suggest a need for further investigation to elucidate potential links between chronic, low-level exposure to algal toxins and immune health.

Liu, D.Y., Keesing, J.K., Dong, Z.J., Zhen, Y., Di, B.P., Shi, Y.J., Fearns, P., and Shi, P. Recurrence of the world's largest green-tide in 2009 in Yellow Sea, China: *Porphyra yezoensis* aquaculture rafts confirmed as nursery for macroalgal blooms. *Marine Pollution Bulletin* 60(9): 1423-1432, 2010.

Notes: In the summer of 2008, the world's largest green-tide occurred in the Yellow Sea, China. The hypothesized cause was the expansion of *Porphyra yezoensis* aquaculture along the Jiangsu coastline and the re-occurrence of a green-tide in 2009 was predicted. In this study, satellite and field images showed the formation of the June 2009 green-tide which again originated from the Jiangsu coast. The responsible species, its source and biomass accumulation were studied to support the previous hypothesis. Morphological and phylogenetic analysis demonstrated the homology of *Ulva prolifera* in the 2008 green-tide with the *U. prolifera* from *P. yezoensis* aquaculture rafts. About 91-505 kg/ha of *U. prolifera* was attached to the *P. yezoensis* aquaculture rafts and a total biomass of 4956 tonnes was estimated during the harvesting of *P. yezoensis*. This is sufficient to seed a bloom when they are dislodged from the rafts as a result of harvesting practices.

Lundholm, N., Clarke, A., and Ellegaard, M. A 100-year record of changing *Pseudo-nitzschia* species in a sill-fjord in Denmark related to nitrogen loading and temperature. *Harmful Algae* 9(5): 449-457, 2010.

Notes: We present a detailed record of shifts in species composition of the toxic diatom genus *Pseudo-nitzschia* from 1905 to 2001 in Mariager Fjord, Denmark, most probably driven by changes in nitrogen loading to the coastal environment and increasing temperatures in the sea. The fertile Mariager Fjord is a long narrow sill-fjord with a permanently anoxic basin. The fjord has experienced increased nutrient loading during the last 100 years and is presently highly eutrophic. 210-Pb-dated sediment cores from the anoxic basin in Mariager Fjord were used in a palaeoecological study to explore changes in species composition. Within the genus *Pseudo-nitzschia* we observed a change in species composition where *Pseudo-nitzschia multiseries* was dominant before 1947, when a shift occurred towards dominance by *Pseudo-nitzschia pungens*. At the same time an increasing relative abundance of *Pseudo-nitzschia seriata* and *Pseudo-nitzschia americana* was observed. The increase in relative abundance of *P. pungens* was positively correlated to nitrogen loading and suggests that increased nitrogen loading during the last 100 years contributes to the observed shift. We hypothesise that increased water temperature in the area could also have contributed to the observed shift in species composition. Three of the species identified (*P. heimii*, *P. americana*, *P. pseudodelicatissima*) are new records for Denmark and Scandinavia. One of these species, *P. americana*, has been linked to increased ammonium concentrations. The preservation of the frustules was excellent and valves of *Pseudo-nitzschia* were identified to species level in sediment dating as far back as 7900 years before present.

Brand, L.E., Pablo, J., Compton, A., Hammerschlag, N., and Mash, D.C. Cyanobacterial blooms and the occurrence of the neurotoxin, beta-N-methylamino-L-alanine (BMAA), in South Florida aquatic food webs. *Harmful Algae* 9(6): 620-635, 2010.

Notes: Recent studies demonstrate that most cyanobacteria produce the neurotoxin beta-N-methylamino-L-alanine (BMAA) and that it can biomagnify in at least one terrestrial food chain. BMAA has been implicated as a significant environmental risk in the development of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS).We examined several blooms of cyanobacteria in South Florida, and the BMAA content of resident animals, including species used as human food. A wide range of BMAA concentrations were found, ranging from below assay detection limits to approximately 7000 mg/g, a concentration associated with a potential long-term human health hazard.

Fu, F.-X., Place, A.R., Garcia, N.S., and Hutchins, D.A. CO_2 and phosphate availability control the toxicity of the harmful bloom dinoflagellate *Karlodinium veneficum*. *Aquatic Microbial Ecology* 59(1): 55-65, 2010.

Notes: We demonstrated that the toxicity of the harmful bloom dinoflagellate *Karlodinium veneficum* is regulated by both CO_2 concentrations and phosphate availability. Semi-continuous cultures were grown in a factorial experiment under all combinations of 3 CO_2 levels (230, 430 and 745 ppm) and 2 phosphate conditions (0.5 and 20 uM). After steady-state acclimation was achieved, karlotoxin cellular quotas and growth rates were determined in all 6 treatments. This strain produced both types of karlotoxin, Km Tx-1 and Km Tx-2. Chlorophyll *a*-normalized production of the 2 types of karlotoxins was much higher in P-limited cultures compared with P-replete ones under the same CO_2 conditions. Increasing CO_2 strongly stimulated production of Km Tx-1 and decreased production of Km Tx-2 in both treatments, but especially in P-limited cultures. Because the Km Tx-1 toxin is an order of magnitude more potent than Km Tx-2, total cellular toxicity was increased dramatically at high pCO_2, particularly in P-limited cultures. Specific growth rates were accelerated by enriched CO_2 in P-replete cultures, but not in P-limited treatments. Growth rates or toxicity of *K. veneficum* could increase substantially in the future with high CO_2 levels in the ocean, depending on P availability, and so interactions between rising CO_2 and eutrophication could cause major shifts in present clay patterns of harmful algal toxin production. These results suggest that over the coming decades, rising CO_2 could substantially increase karlotoxin damage to food webs in the often P-limited estuaries where *Karlodinium* blooms occur.

Kamiyama, T., Nagai, S., Suzuki, T., and Miyamura, K. Effect of temperature on production of okadaic acid, dinophysistoxin-1, and pectenotoxin-2 by *Dinophysis acuminata* in culture experiments. *Aquatic Microbial Ecology* 60(2): 193-202, 2010.

Notes: We cultivated *Dinophysis acuminata*, a mixotrophic dinoflagellate causing diarrhetic shellfish poisoning worldwide, at different temperatures by providing a mixotrophic ciliate *Myrionecta rubra* as a food source. We examined the effects of temperature on growth rates and monitored production of the toxins okadaic acid (OA), dinophysistoxin-1 (DTX1), and pectenotoxin-2 (PTX2) using liquid chromatography-tandem mass spectrometry. Cell densities of *D. acuminata* increased at temperatures of 10, 14, 18, and 22 ºC, and the mean specific growth rates during the exponential growth phase were higher at higher temperatures (0.14 to 0.28 d^{-1}). The concentration of all toxins increased as cell densities increased. The mean cellular PTX2 content during the exponential growth phase was greater at lower temperatures, but clear differences in the other toxin concentrations in relation to temperature were not observed. The cellular content of OA and DTX1 significantly increased during the exponential growth phase at all temperatures except at 10 ºC for OA, whereas that of PTX2 did not significantly increase at temperatures greater than 10 ºC. Cellular toxin production rates temporarily increased at or just before the end of the exponential growth phase at 14, 18, and 22 ºC, and the mean rates for OA production over the entire incubation period were higher at higher temperatures. Our results indicate that increasing temperature generally stimulates toxin production in *D. acuminata* populations due to an increase in cell density, but cellular content and production rates of OA and PTX2 in response to temperature differ and are influenced by growth phase.

Saoudi, M., Abdelmouleh, A., and El Feki, A. Tetrodotoxin: a potent marine toxin. *Toxin Reviews* 29(2): 60-70, 2010.

Notes: Tetrodotoxin (TTX) is a neurotoxin found in puffer fish and other marine animals. This toxin is predominantly isolated from the skin, viscera, ovaries, and liver of the puffer fish. The toxin is produced by various species of bacteria, and TTX-bearing animals may have absorbed and accumulated it through the food chain. TTX is widely used in many laboratories as an important pharmacological reagent because of its ability to selectively block the sodium channels on the nerve membrane. No antidote is available for clinical use. The mainstay of treatment is careful observation and serial neurological assessment to monitor the progression of the clinical effects so that respiratory failure or cardiac effects are appropriately treated.

Bownik, A. Harmful algae: effects of alkaloid cyanotoxins on animal and human health. *Toxin Reviews* 29(3-4): 99-114, 2010.

Notes: Cyanobacteria are bloom-forming procaryotic microorganisms producing cyanotoxins – secondary metabolites toxic to aquatic and terrestial animals and also humans. Alkaloid cyanotoxins are: neurotoxic anatoxin-a, saxitoxin and cytotoxic cylindrospermopsin, which inhibits protein synthesis in various cell types and neurotoxic saxitoxin. These substances are very harmful to many animal species. Moreover, they may accumulate at high concentrations in various tissues of aquatic animals such as bivalves and fish, which can be a source of intoxication for predators and humans. This review presents the current state of knowledge on the effects of alkaloid cyanotoxins on different animal species and human health.

Oshiro, N., Yogi, K., Asato, S., Sasaki, T., Tamanaha, K., Hirama, M., Yasumoto, T., and Inafuku, Y. Ciguatera incidence and fish toxicity in Okinawa, Japan. *Toxicon* 56(5): 656-661, 2010.

Notes: Okinawa being located in the subtropical region has the highest incidence of ciguatera in Japan. Officially, 33 outbreaks involving 103 patients have been reported between 1997 and 2006. The implicated species were *Variola louti*, *Lutjanus bohar*, *Lutjanus monostigma*, *Epinephelus fuscoguttatus*, unidentified *Lutjanus* sp., *Plectropomus areolatus*, *Oplegnathus punctatus*, *Epinephelus polyphekadion*, *Caranx ignobilis* and moray eel. Toxicities of the leftover meals, as determined by mouse bioassays, ranged from 0.025 to 0.8 MU/g or above (equivalent to 0.175 - 5.6 ng CTX1B/g). We collected 612 specimens of fish belonging to *L. monostigma*, *L. bohar*, *Lutjanus argentimaculatus*, *Lutjanus russellii* , *V. louti*, *Variola albimarginata*, and *E. fuscoguttatus* from the coasts around Okinawa and examined the toxicity of the flesh by the mouse bioassay. The rate of toxic fish was as follows: *L. monostigma*: 32.3%, *L. bohar*: 11.9%, *V. louti*: 14.3%, *E. fuscoguttatus*: 20.8%. Only one out of 36 samples of *V. albimarginata* and two of 74 samples of *L. russellii* were found toxic. None of the 35 samples of *L. argentimaculatus* was toxic. Nor the *L. bohar* samples weighing less than 4 kg were toxic. In all toxic samples, CTX1B was detected by LC/MS analysis but CTX3C and 51-hydroxyCTX3C were not.

Baumann, F., Bourrat, M.-B., and Pauillac, S. Prevalence, symptoms and chronicity of ciguatera in New Caledonia: Results from an adult population survey conducted in Noumea during 2005. *Toxicon* 56(5): 662-667, 2010.

Notes: Ciguatera is a widespread ichthyosarcotoxism which causes gastrointestinal, neurological and cardiovascular disturbances. Investigations conducted by ORSTOM in 1992 highlighted a prevalence of 25% in the adult population of Noumea, New Caledonia. The main objective of our study was to estimate the prevalence of ciguatera and the persistence of symptoms by sex and by ethnicity among adult patients of a nurse clinic in Noumea in 2005. Investigations were conducted from 1st January to 15th June 2005. During this period, 559 patients were included: 165 males and 394 females. Among them, 37.8% were poisoned at least once in their life. This rate was independent of gender and ethnicity, but was significantly higher in age groups above 40 years. Neurological signs were more frequent (>80%) than gastrointestinal (<50%) and cardiac signs (<15%). Symptoms presented no difference between ethnic or gender groups, even for subjective signs. Most poisonings were due to carnivorous fishes, but quite all species living in the lagoon were quoted. Symptoms persisted more than one year for 34% of the population, in both Melanesians and Caucasians. This study shows a significant increase of ciguatera prevalence, and its chronicity for 1/5 of European cases.

Hamilton, B., Whittle, N., Shaw, G., Eaglesham, G., Moore, M.R., and Lewis, R.J. Human fatality associated with Pacific ciguatoxin contaminated fish. *Toxicon* 56(5): 668-673, 2010.

Notes: Ciguatera is a food poisoning identified as the principal risk factor in the consumption of tropical fish in Oceania. The syndrome, which follows ingestion of ciguatoxin-contaminated ciguateric fishes, is characterised by an array of gastrointestinal and neurological features. In this report we examine forensic samples associated with a human fatality using a 3H-brevetoxin binding assay and reversed-phase HPLC/MS and HPLC/MS/MS. Three Pacific ciguatoxins (P-CTX) were detected in the implicated fish flesh sample by LC-MS/MS, implicating multiple P-CTXs in the fatal case. Additionally, ciguatoxin was identified in a liver sample obtained at post-mortem. The level of ciguatoxin detected (0.14 ppb P-CTX-1 equivalents by binding assay) indicated that at least 10% of the ingested P-CTX-1 remained in the human liver 6 days after the toxic fish was consumed. This study confirms the potential of tropical reef fish to accumulate sufficient P-CTX to be lethal to humans, especially if the liver and viscera are consumed as part of the meal.

Chinain, M., Darius, H.T., Ung, A., Fouc, M.T., Revel, T., Cruchet, P., Pauillac, S., and Laurent, D. Ciguatera risk management in French Polynesia: The case study of Raivavae Island (Australes Archipelago). *Toxicon* 56(5): 674-690, 2010.

Notes: Based on epidemiological data available through long-term monitoring surveys conducted by both the Public Health Directorate and the Louis Malardé Institute, ciguatera is highly endemic in French Polynesia, most notably in Raivavae (Australes) which appears as a hot spot of ciguatera with an average incidence rate of 140 cases/10,000 population for the period 2007-2008. In order to document the ciguatera risk associated with Raivavae lagoon, algal and toxin-based field monitoring programs were conducted in this island from April 2007 to May 2008. Practically, the distribution, abundance and toxicity of *Gambierdiscus* populations, along with the toxicity levels in 160 fish distributed within 25 distinct species, were assessed in various sampling locations. Herbivores such as Scarids (parrotfish) and Acanthurids (unicornfish) were rated as high-risk species based on receptor-binding assay toxicity data. A map of the risk stratification within the Raivavae lagoon was also produced, which indicates that locations where both natural and man-made disturbances have occurred remained the most susceptible to CFP incidents. Our findings also suggest that, locally, the traditional knowledge about ciguatera may not be scientifically complete but is functionally correct. Community education resulted in self-regulating behaviour towards avoidance of high-risk fish species and fishing locations.

Llewellyn, L.E. Revisiting the association between sea surface temperature and the epidemiology of fish poisoning in the South Pacific: Reassessing the link between ciguatera and climate change. *Toxicon* 56(5): 691-697, 2010.

Notes: The most detailed dataset of ciguatera intensity is that produced by the South Pacific Epidemiological and Health Information Service (SPEHIS) of the Secretariat of the Pacific Community. The SPEHIS fish poisoning database has been previously analysed yielding statistically significant correlations between the Southern Oscillation Index (SOI) and ciguatera case numbers in several countries raising concerns this affliction will increase as oceans warm. Mapping of the SPEHIS records and other data hints at ciguatera not only being restricted to warm waters but that the Indo-Pacific Warm Pool, a body of water that remains hot throughout much of the year, may inhibit ciguatera prevalence. A qualitative assessment of ciguatera intensity and sea surface temperature (SST) behaviour within the EEZ of selected South Pacific nations supported the notion that ciguatera intensity was highest when SST was between an upper and lower limit. Many more climate and SST indices beyond the SOI are now available, including some that measure the abovementioned phenomenon of oceanic warm pools. Statistically significant, positive and negative cross-correlations were obtained between time series of annual ciguatera case rates from the SPEHIS dataset and the Pacific Warm Pool Index and several ENSO related indices which had been lagged for up to 2 years before the ciguatera time series. This further supports the possibility that when considering the impact of climate change on ciguatera, one has to consider two thresholds, namely waters that remain warm enough for a long enough period can lead to ciguatera and that extended periods where the water remains too hot may depress ciguatera case rates. Such a model would complicate projections of the effects of climate change upon ciguatera beyond that of a simple relationship where increased SST may cause more ciguatera.

Tester, P.A., Feldman, R.L., Nau, A.W., Kibler, S.R., and Wayne, L.R. Ciguatera fish poisoning and sea surface temperatures in the Caribbean Sea and the West Indies. *Toxicon* 56(5): 698-710, 2010.

Notes: Ciguatera fish poisoning (CFP) is a circumtropical disease caused by ingestion of a variety of reef fish that bioaccumulate algal toxins. Distribution and abundance of the organisms that produce these toxins, chiefly dinoflagellates of the genus *Gambierdiscus*, are reported to correlate positively with water temperature. Consequently, there is growing concern that increasing temperatures associated with climate change could increase the incidence of CFP. This concern prompted experiments on the growth rates of six G*ambierdiscus* species at temperatures between 18°C and 33°C and the examination of sea surface temperatures in the Caribbean and West Indies for areas that could sustain rapid *Gambierdiscus* growth rates year-round. The thermal optimum for five of six *Gambierdiscus* species tested was ≥29°C. Long-term SST data from the southern Gulf of Mexico indicate the number of days with sea surface temperatures ≥29°C has nearly doubled (44 to 86) in the last three decades. To determine how the sea surface temperatures and *Gambierdiscus* growth data correlate with CFP incidences in the Caribbean, a literature review and a uniform, region-wide survey (1996-2006) of CFP cases were conducted. The highest CFP incidence rates were in the eastern Caribbean where water temperatures are warmest and least variable.

Litaker, R.W., Vandersea, M.W., Faust, M.A., Kibler, S.R., Nau, A.W., Holland, W.C., Chinain, M., Holmes, M.J., and Tester, P.A. Global distribution of ciguatera causing dinoflagellates in the genus *Gambierdiscus*. *Toxicon* 56(5): 711-730, 2010.

Notes: Dinoflagellates in the genus *Gambierdiscus* produce toxins that bioaccumulate in tropical and sub-tropical fishes causing ciguatera fish poisoning (CFP). Little is known about the diversity and distribution of *Gambierdiscus* species, the degree to which individual species vary in toxicity, and the role each plays in causing CFP. This paper presents the first global distribution of *Gambierdiscus* species. Phylogenetic analyses of the existing isolates indicate that five species are endemic to the Atlantic (including the Caribbean/West Indies and Gulf of Mexico), five are endemic to the tropical Pacific, and that two species, *Gambierdiscus carpenteri* and *Gambierdiscus caribaeus* are globally distributed. The differences in *Gambierdiscus* species composition in the Atlantic and Pacific correlated with structural differences in the ciguatoxins reported from Atlantic and Pacific fish. This correlation supports the hypothesis that *Gambierdiscus* species in each region produce different toxin suites. A literature survey indicated a >100-fold variation in toxicity among species compared with a 2 to 9-fold within species variation due to changing growth conditions. These observations suggest that CFP events are driven more by inherent differences in species toxicity than by environmental modulation. How variations in species toxicity may affect the development of an early warning system for CFP is discussed.

Rhodes, L.L., Smith, K.F., Munday, R., Selwood, A.I., McNabb, P.S., Holland, P.T., and Bottein, M.Y. Toxic dinoflagellates (Dinophyceae) from Rarotonga, Cook Islands. *Toxicon* 56(5): 751-758, 2010.

Notes: Dinoflagellate species isolated from the green calcareous seaweed, *Halimeda* sp. J.V. Lamouroux, growing in Rarotongan lagoons, included *Gambierdiscus australes* Faust & Chinain, *Coolia monotis* Meunier, *Amphidinium carterae* Hulburth, *Prorocentrum lima* (Ehrenberg) Dodge, *P.* cf. *maculosum* Faust and species in the genus *Ostreopsis* Schmidt. Isolates were identified to species level by scanning electron microscopy and/or DNA sequence analysis. Culture extracts of *G. australes* isolate CAWD149 gave a response of 0.04 pg P-CTX-1 equiv. per cell by an N2A cytotoxicity assay (equivalent to ca 0.4 pg CTX-3C $cell^{-1}$). However, ciguatoxins were not detected by LC-MS/MS. Partitioned fractions of the cell extracts potentially containing maitotoxin were found to be very toxic to mice after intraperitoneal (i.p.) injection. *A. carterae* was also of interest as extracts of mass cultures caused respiratory paralysis in mice at high doses, both by i.p. injection and by oral administration. The Rarotongan isolate fell into a different clade to New Zealand *A. carterae* isolates, based on DNA sequence analysis, and also had a different toxin profile. As *A.carterae* co-occurred with *G. australes*, it may contribute to human poisonings attributed to CTX and warrants further investigation. A crude extract of *C. monotis* was of low toxicity to mice by i.p. injection, and an extract of *Ostreopsis* sp. was negative in the palytoxin haemolysis neutralisation assay.

Aráoz, R., Molgó, J., and Tandeau de Marsac, N. Neurotoxic cyanobacterial toxins. *Toxicon* 56(5): 813-828, 2010.

Notes: Worldwide development of cyanobacterial blooms has significantly increased in marine and continental waters in the last century due to water eutrophication. This phenomenon is favoured by the ability of planktonic cyanobacteria to synthesize gas vesicles that allow them to float in the water column. Besides, benthic cyanobacteria that proliferate at the bottom of lakes, rivers and costal waters form dense mats near the shore. Cyanobacterial massive proliferation is of public concern regarding the capacity of certain cyanobacterial strains to produce hepatotoxic and neurotoxic compounds that can affect public health, human activities and wild and stock animals. The cholinergic synapses and voltage-gated sodium channels constitute the targets of choice of cyanobacterial neurotoxins. Anatoxin-a and homoanatoxin-a are agonists of nicotinic acetylcholine receptors. Anatoxin-a(s) is an irreversible inhibitor of acetylcholinesterase. Saxitoxin, kalkitoxin and jamaicamide are blockers of voltage-gated sodium channels, whereas antillatoxin is an activator of such channels. Moreover the neurotoxic amino acid L-beta-*N*-methylamino-L-alanine was shown to be produced by diverse cyanobacterial taxa. Although controversial, increasing *in vivo* and *in vitro* evidence suggest a link between the ingestion of L-beta-*N*-methylamino-L-alanine and the development of amyotrophic lateral sclerosis/Parkinsonism-dementia complex, a neurodegenerative disease. This paper reviews the occurrence of cyanobacterial neurotoxins, their chemical properties, mode of action and biosynthetic pathways.

Méjean, A., Peyraud-Thomas, C., Kerbrat, A.S., Golubic, S., Pauillac, S., Chinain, M., and Laurent, D. First identification of the neurotoxin homoanatoxin-a from mats of *Hydrocoleum lyngbyaceum* (marine cyanobacterium) possibly linked to giant clam poisoning in New Caledonia. *Toxicon* 56(5): 829-835, 2010.

Notes: We report the first identification of homoanatoxin-a from benthic marine cyanobacteria (*Hydrocoleum lyngbyaceum*) samples collected in Lifou (Loyalty Islands, New Caledonia), where cases of giant clams (*Tridacna maxima*) intoxications were recorded during a severe ciguatera fish poisoning outbreak. Homoanatoxin-a was also detected in extracts of giant clams harvested in the surroundings of the contaminated area suggesting the possible link between these poisoning events and the occurrence of potentially neurotoxic *Hydrocoleum*.

Golubic, S., Abed, R.M.M., Palinska, K., Pauillac, S., Chinain, M., and Laurent, D. Marine toxic cyanobacteria: Diversity, environmental responses and hazards. *Toxicon* 56(5): 836-841, 2010.

Notes: Toxic cyanobacterial blooms have been a primary concern predominantly in the plankton of freshwater bodies. Recently, however, the toxicity of benthic cyanobacteria is increasingly attracting attention of the scientific community and environmental agencies. The occurrence of toxic strains in benthic cyanobacteria is intimately linked to our understanding of the diversity and ecological responses of these organisms under field conditions. To that effect, we are engaged in combined morphotypic and genotypic characterization (polyphasic) of benthic natural populations of cyanobacteria in tropical lagoons and coral reefs, with the objective to provide a reliable reference for further comparative work. The methods of identification based on phenotypic properties and those based on molecular tools for genotypic identification are correlated. The approach is based on identifying the occurrences of cyanobacterial benthic blooms, tested for purity and analyzed by application of molecular tools. The questions addressed include the distinction between marine and freshwater taxa, between populations in geographically separate regions as well as between their potential vs. expressed toxicity.

Louzao, M.C., Espiña, B., Cagide, E., Ares, I.R., Alfonso, A., Vieytes, M.R., and Botana, L.M. Cytotoxic effect of palytoxin on mussel. *Toxicon* 56(5): 842-847, 2010.

Notes: Palytoxin is a large and complex polyhydroxylated molecule with potent neurotoxic activity. Dinoflagellates from the *Ostreopsis* genera were demonstrated to be producers of this compound and analogues. Even though initially palytoxin appearance was restricted to tropical areas, the recent occurrence of *Ostreopsis* outbreaks in the Mediterranean Sea point to a worldwide dissemination probably related to climatic change. Those dinoflagellates can bioaccumulate in shellfish, especially in filter-feeding mollusks and have been involved in damaging effects in seafood or human toxic outbreaks. The present study describes palytoxins effect on metabolic activity of mantle and hepatopancreas cells from the mussel *Mytilus galloprovincialis* Lmk. Our results indicate that palytoxin is highly cytotoxic to mussel cells; unlike what happens with other toxins more

common in European coasts such as okadaic acid and azaspiracid. These findings have a special significance for the marine environment and aquaculture since there is evidence for the ability of palytoxin to affect the integrity of bivalve mollusks that are not adapted to the presence of this toxin.

Boada, L.D. *et al.* Ciguatera fish poisoning on the West Africa Coast: An emerging risk in the Canary Islands (Spain). *Toxicon* 56(8): 1516-1519, 2010.

Notes: Ciguatera fish poisoning (CFP) is endemic in certain tropical and subtropical regions of the world. CFP had not been described on the West Africa Coast until a 2004 outbreak in the Canary Islands. In 2008-2009, two additional outbreaks of ciguatera occurred. Individuals afflicted had consumed lesser amberjack (*Seriola rivoliana*) captured from nearby waters. Caribbean ciguatoxin-1 (C-CTX-1) was confirmed in fish samples by LC-MS/MS. Ciguatoxic fish in this region may pose a new health risk for the seafood consumer.