# Harmful Algae News

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## Genomic resources for the domoic acid-producing diatom *Pseudo-nitzschia multistriata*

Species responsible for Harmful Algal Blooms (HABs) are among the best studied unicellular microalgae. HABs pose a serious risk to human health and are responsible for considerable economic losses in the aquaculture industry which has resulted in the funding of monitoring programs to investigate their temporal and spatial distribution, as well as research projects aimed at understanding various aspects of their biology. In recent decades, understanding about HAB diversity, physiology and trophic habits, the mechanisms that regulate toxin production, and the role of environmental variables and interactions with other members of the plankton community on their growth dynamics has increased. The rapid development of genomic- and transcriptomic-based approaches, now available for an increasing number of unicellular eukaryotes including HAB species, is allowing scientists to gain a mechanistic understanding of a broad range of biological features.

The genus *Pseudo-nitzschia* includes the majority of species that produce the neurotoxin domoic acid, the causative agent of Amnesic Shellfish Poisoning. About 50 species have been described to date and half of these are known to produce domoic acid. The importance of this genus motivated the selection of one of the most toxic species, *Pseudo-nitzschia multiseries*, to be one of the few

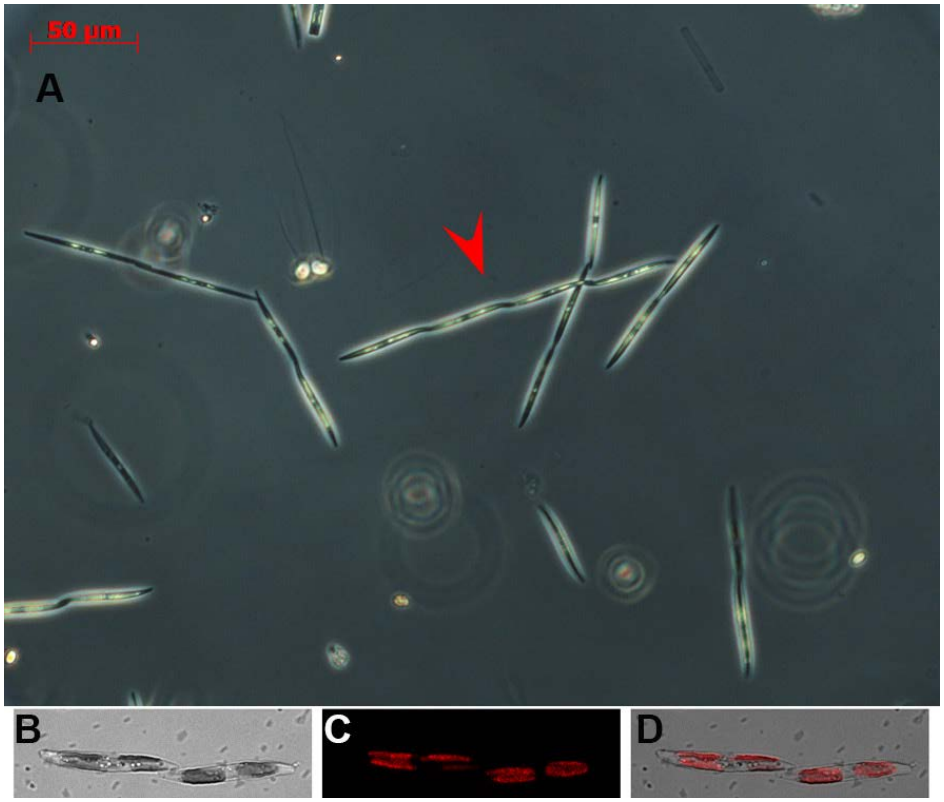


*Fig. 1. A. Light micrograph of a natural phytoplankton sample collected at the Long Term Ecological Research station MareChiara (Gulf of Naples, Italy) on October 3rd 2013. Pseudo-nitzschia multistriata cells (a chain is indicated with an arrow) can be easily identified by their slightly sigmoid shape. Photo: Marina Montresor. Two Pseudo-nitzschia multistriata cells approaching cell division, B. bright field, C. chlorophyll autofluorescence in the chloroplasts, and D. merged images.*

### Content

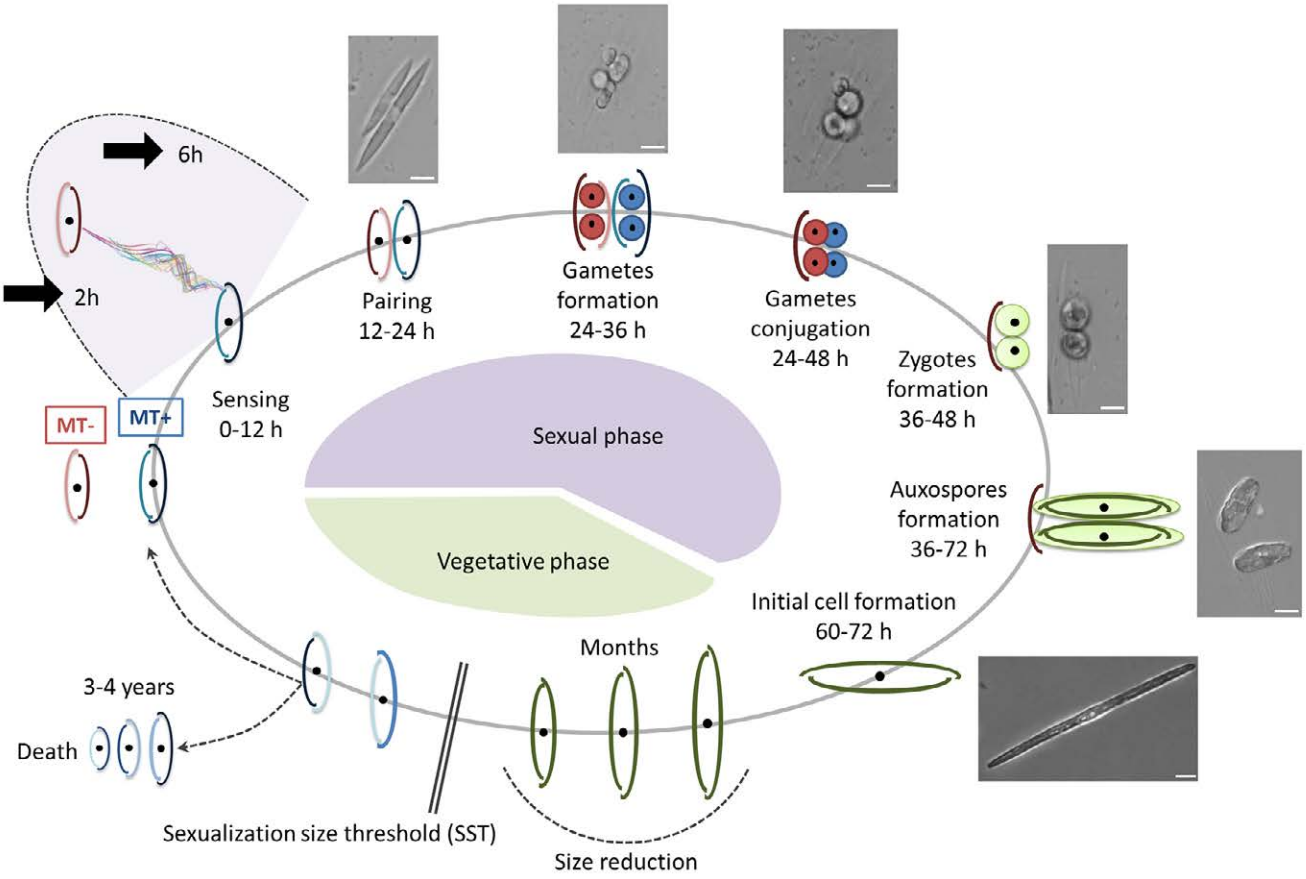


*Fig. 2. Schematic drawing of the life cycle of the pennate diatom Pseudo-nitzschia multistriata. The vegetative phase is characterized by progressive cell size reduction of the population. When cells reach the sexual size threshold (SST), they can either keep decreasing in size until they die, or undergo sexual reproduction and escape the size-decreasing process producing large initial cells. The perception of chemical cues deriving from the mating partner brings cells of opposite mating type to pair and haploid gametes are produced following meiosis. Conjugation of gametes produces two expandable zygotes that develop into auxospores, within which an initial cell of maximum size is synthesized, restoring the vegetative phase of the cycle. Representative microscopic images of the different stages are shown outside the circle; bar, 10 µm. From [7].*

diatoms selected for genome sequencing when this was still a demanding and time consuming effort. The *P. multiseries* genome has been publicly available since 2011 (*https://mycocosm.jgi.doe.gov/Psemu1/Psemu1.home.html*) and despite being quite fragmented, possibly due to the high content of repetitive sequences it has represented a valuable resource used to explore diatom biology in general and in many comparative genomic analyses. Subsequently, *Pseudo-nitzschia* transcriptomes became available through the Marine Microbial Eukaryotic Transcriptome Sequencing Project (MMETSP), enlarging the repertoire of sequence resources for the genus (*https://www.imicrobe.us/#/search/mmetsp*). Currently, thanks to this initiative, transcriptomics data are available for *P. arenysensis*, *P. delicatissima*, *P. fraudulenta*, *P. australis*, *P. heimii* and *P. pungens*. Transcriptomics data have also been generated in dedicated studies for other species, namely *P. multiseries, P. granii, P. seriata* and *P. obtusa* [1-3].

Here we illustrate genomic resources that are available to the research community for another toxic *Pseudo-nitzschia* species, *P. multistriata.* We also provide a few examples of questions that can be addressed by genomic approaches.

*Pseudo-nitzschia multistriata* was described in 1993 by Hideaki Takano from coastal waters in southern Japan. The species produces domoic acid [4] and has been reported from various regions world-wide (Mediterranean Sea, Gulf of Mexico, Malaysia, Singapore, New Zealand, the Pacific coast of Mexico, see [5]). Cells of *P. multistriata* can easily be identified in light microscopy due to their distinct 'sigmoid' shape (Figure 1). This species began to be recorded at the Long Term Ecological Research station Mare Chiara in the Gulf of Naples in 1993 and since then is regularly recorded in summer-early autumn. The relative ease of identification made it a model for a long term study of population structure using microsatellites [6]. This study required the isolation of a few hundred strains to obtain their DNA. Availability of all of these strains represented a great resource facilitating the use of *P. multistriata* as a genetic model, allowing genetic crosses and production of new generations in the laboratory.

We would like to avail of this opportunity to publicize our request for *P. multistriata* strains to all of our colleagues working with phytoplankton samples who may find the species, easily distinguishable from other *Pseudo-nitzschia* due to the curved tips and sigmoid shape. Strains from different geographic locations will greatly enrich ongoing population genomics analyses and could reveal more about core and dispensable regions of the genome, selection, and ultimately about the evolution of this toxic species.


*Fig. 3. The genome browser available on the SZN BioInforma platform.*

The genome could be sequenced exploiting inbred strains, obtained from the cross of a first generation of sibling strains [7]. Because of the lower polymorphism of inbreds, it was possible to reconstruct long fragments of DNA from reads obtained with the Illumina technology, overcoming the limits of short reads assembly. Indeed, the assembly of high polymorphic sequence reads make it harder for common assembly software to bind small sequences together to produce the long fragments called scaffolds.

The 59 Mb genome obtained enhanced the range of possible approaches to explore the genetic basis of many of the species features, including the capability to undergo sexual reproduction. *P. multistriata*, like the majority of pennate diatoms, has a heterothallic mating system. This implies that sexual reproduction, the process in diatoms that counteracts progressive cell size reduction to produce large-sized F1 cells, only occurs when cells of opposite mating type (MT+ and MT-) make contact (Figure 2) [7]. Gene expression studies based on RNA-seq have focused on the life cycle and on the sexual reproduction phase, revealing differences in the transcriptomic behavior between the two mating types, even if they are morphologically identical. One of the most exciting findings enabled by the genome sequence availability was the discovery of the first mating type determining gene for diatoms. How a cell becomes MT+ or MT- was unknown; differential expression of a specific gene between groups of MT+ and MT- strains was linked to structural differences in a specific genomic region, providing the first clue on how the MT is specified [8].

Diatom genomes, when compared to estimates for other toxic phytoplankton such as dinoflagellate species, are generally smaller and less "intimidating", and genome sequencing is approachable for even small teams, without the requirement to establish large consortia. The *P. multistriata* genome sequencing project was a small project funded by a Marie Curie Career integration grant, it mainly involved two teams, the SZN in Naples (Italy) and the TGAC in Norwich (UK) (now Earlham Institute), cost around 10,000 euros and was initially released on a TGAC browser which was password protected in line with institute policies. To make it more easily accessible, the genome browser is now being hosted by the bioinformatics service at the SZN, (*http://bioinfo.szn.it/pmultistriata/*), embedded in the service platform (*http://bioinfo.szn.it/*; Figure 3) where it is freely accessible together with all the related RNA-seq data produced so far. Beyond gene tags, researchers have the possibility to browse the genome information visualizing repetitive elements, conserved regions in common with other species and other features, with links provided to the data files and to the relevant literature. Finally, within the European project EMBRIC and with the assistance of European Bioinformatics Institute teams, additional work on the sequencing data has been done to have it released in Ensembl (*https://protists.ensembl.org/Pseudonitzschia_multistriata/Info/Index*), one of the major genome browsers, originally created for vertebrate genomes but with an expanding section for protists. Availability in Ensembl makes the data readily discoverable by the HAB community, by scientists working on very distantly related organisms, and facilitates its use in large scale comparative studies. Unfortunately due to specific requirements of the Ensembl browser which did not support the original files generated for *P. multistriata*, the SZN and the Ensembl versions of the genome are not uniform; the scaffold names are different and, in Ensembl, all *P. multistriata* proteins are reported as "unknown proteins" while a detailed functional annotation is provided by the platform available from SZN.

While these discrepancies highlight the existence of difficulties in crosslinking –omics data from different resources, especially for non-model systems as well as the need for constant and supported interaction between wet lab scientists and bioinformaticians, the availability of *P. multistriata* genome resources was a big opportunity for comparative analyses. It has enabled and strengthened important discoveries, such as the conservation of a cluster of four genes involved in domoic acid synthesis, originally identified in the *P. multiseries* genome [9].

*Continued on page 5*

*Fig. 4. Some Pseudo-nitzschia fans at Stazione Zoologica Anton Dohrn: (standing from left to right) Svenja Mager, Maria Immacolata Ferrante , Francesco Manfellotto, Anna Santin, Maria Valeria Ruggiero, Monia Russo, Viviana Di Tuccio, (sitting from right to left) Rossella Annunziata, Antonella Ruggiero, Pina Marotta, Marina Montresor*

# When tides collide: Harmful cyanobacterial and microalgal blooms in Florida and implications for risk assessment

Cyanobacterial blooms are a regular occurrence in southern Florida. Water releases from Lake Okeechobee to maintain the water level in this large lake regularly occur along the St. Lucie Canal to the eastern seaboard or along the Caloosahatchee to Fort Myers and the Gulf of Mexico (Fig. 1). Due to increased nutrient loading in Lake Okeechobee, cyanobacterial blooms, frequently composed of *Microcystis aeruginosa* are released from the lake and contaminate both these waterways. This results in large, microcystin-containing cyanobacterial blooms that can affect tourism and fisheries (Fig. 2). Press reports cataloguing these economic and health impacts in Florida are common.

In summer 2018, the situation on the west coast of Florida was exacerbated by the appearance of a bloom of *Karenia brevis* in the Gulf of Mexico. This brevetoxin-producing algal bloom resulted in the deaths of marine mammals and fish with numbers in the thousands reported on the beaches around southwest Florida. Due to the large discharge of water from Lake Okeechobee, pulses of freshwater ended up in the estuarine and coastal environments.

Sampling of the Caloosahatchee in 2018 showed high concentrations of *Microcystis aeruginosa* and microcystin-LR according to microscopy, UPLC-PDA and UPLC-MS [1]. BMAA was also detected by triple quadrupole mass spectrometry at much lower concentration. Although such blooms dominated the freshwater environment of the river, visually intact *Microcystis* colonies were observed in brackish water in the sound and estuary past the tide line. Conversely, although diatoms and dinoflagellates could not be observed in freshwater samples, ELISA analysis for brevetoxins showed low positive concentrations in the Caloosahatchee and large concentrations in the brackish and marine environment.

The findings of the "crossing-over" of blooms raise questions concerning the risk assessment of cyanobacterial and dinoflagellate toxins in estuarine environments. First, how far can toxins produced by freshwater cyanobacterial blooms be found in saline environments? Conversely, can marine diatom and dinoflagellate toxins be routinely found in freshwater environments, most likely through navigation and tidal flows? Our data indicate that both are possible, although a greater understanding of the effect of salinity on the growth of *Microcystis* is required, building upon previous work that has shown that e.g. microcystin concentrations can be increased after salt shock [2]. Furthermore, in other estuarine environments a wide range of marine and freshwater toxins have been identified [3].

If the particular toxin(s) are known within freshwater or marine blooms, then adequate risk assessment is possible to protect human and animal health. However, when multiple blooms occur at the same location, then toxicity assessment and risk assessment are necessary to determine whether there are interactions between the toxicants that may change the toxicological outcome. Communication is also essential in order to alert the public to potential issues that may arise from exposure to one or more classes of aquatic toxin(s), whether from harmful cyanobacterial and/or algal blooms. Further communication may be necessary as, depending on the country, marine and freshwaters may be under the jurisdiction of different agencies with different accountabilities and responsibilities. The presence of such multiple blooms may also have effects upstream as, in the case of Florida, changes to discharges from Lake Okeechobee may have to occur when marine blooms are present. When tides collide, procedures need to be in place to determine the potential risk, communicate with relevant parties and perform toxicity assessments when necessary. In addition, collection of autopsy materials can also determine what toxicants may be causing adverse health effects, whether to wild or domestic animals or humans. Ultimately, legislation and good stewardship of waters and relevant contingencies can minimise the potential risk of adverse health effects from occurring.

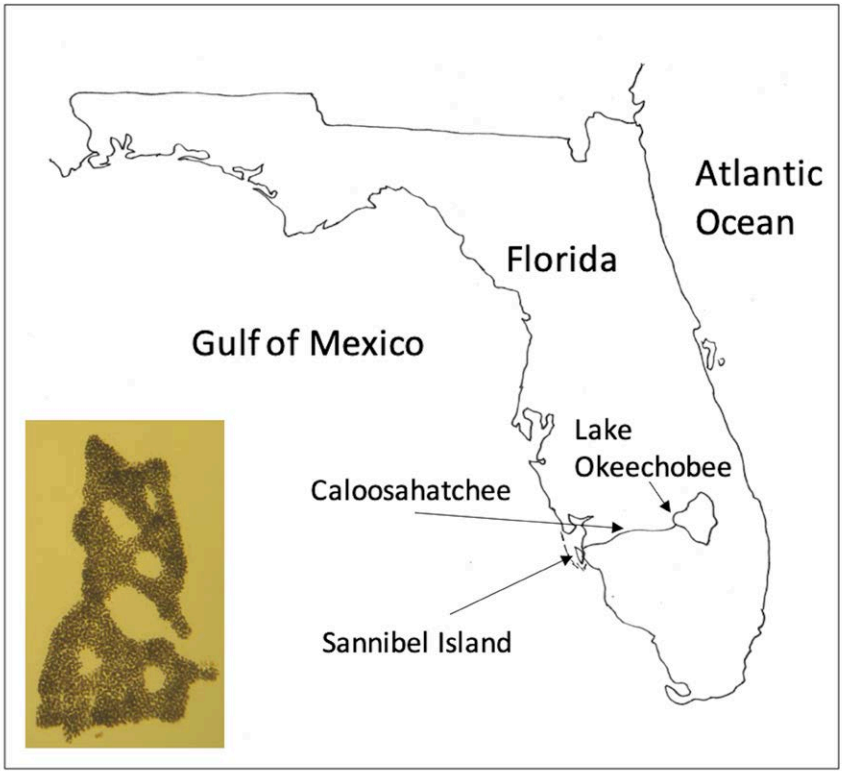


*Fig. 1. Location of Lake Okeechobee and Caloosahatchee River in Southern Florida, USA where a bloom of Microcystis aeruginosa released from the lake collided with a red tide of brevetoxin-producing Karenia brevis near Sannibel Island. Bottom left Insert, a colony of Microcystis aeruginosa (approx. 500 m width) from Caloosahatchee River*


## Acknowledgements

We are grateful to Dr. John Cassini, Jason

*Fig. 2. Bloom of Microcystis aeruginosa at Lake Okeechobee, Florida, summer 2018*

Pim and Calusa Waterkeepers for assistance in sampling.

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The raw and processed sequencing data generated can be beneficial for many applications. Some are already routine methods, such as the use of the genome as a reference to map RNA-seq reads and interpret transcriptomics studies, comparative analyses with other genomes to define structural and/or phylogenetic relationships, estimation of the extent of genomic variations and rearrangements, and the identification of regions under selection, contributing to evolutionary insights in populations and species. Other analyses that can benefit from the existence of a reference genome include support in taxonomic assignation and in the interpretation of results from metagenomics and metatranscriptomics projects. Combining information from such approaches can lead to innovative ways to monitor phytoplankton; for example, genes found highly induced under specific experimental conditions in the laboratory can become markers to follow specific processes in the natural environment by qPCR or metatranscriptomics. Examples include genes involved in the synthesis of toxins, genes expressed during sexual reproduction, genes activated during specific stress conditions. We are now familiar with the fact that community composition can be described with barcode sequences. In the future we will be able to identify organisms by retrieving their genomes, as is now the case for bacteria. Moreover, species-specific genes allow specific functions from individual species to be understood, e.g. to obtain a picture of their metabolic state, or the phase of their life history. Finally, for many traditional or more innovative methods for the study of gene function, the presence of a reference genome is mandatory. Sophisticated manipulations and genome editing methods, including the CRISPR/Cas9 technology, are now within reach for this *Pseudo-nitzschia* species.

In view of the potential for *P. multistriata* to be used as a model for planktonic diatom life cycles, more studies are ongoing, and a further leap forward is expected thanks to the support of the Marine Microbial Initiative of the Gordon and Betty Moore Foundation, which is funding a project (*http://www.szn.it/index.php/en/research/integrative-marine-ecology/research-projects-emi/disco*) to dissect the mechanisms underlying sex determination and controlling transitions between life cycle phases, to discover the genes and genomic regions that are under selection in natural populations, and to assess the effects of sex on genome evolution. More *P. multistriata* genomes are being re-sequenced thanks to this project, and technological advances and new technologies gradually becoming available will be exploited to improve the current genome assembly, to explore epigenetic control and to bring our knowledge of *Pseudo-nitzschia* to a higher functional level.

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# Record levels of Dinophysistoxin-2 in clams from Douarnenez Bay, France, after an unusual bloom of *Dinophysis acuta*

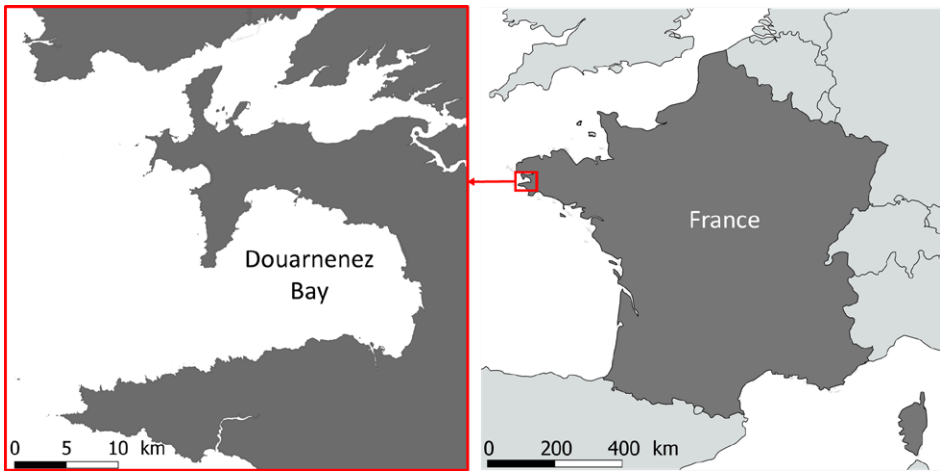


*Fig. 1. Location of Douarnenez Bay (48° 5' 29" North; 4° 19' 51" West), Western French Atlantic coast.*

The official monitoring network for phytoplankton and algal toxins in French shellfish production areas (REPHY) was established by Ifremer in 1984 after several thousand cases of diarrhetic shellfish poisoning occurred in Western France [1]. The monitoring program has evolved over time. From January 1, 2010 chemical analysis by liquid chromatography coupled with mass spectrometery in tandem (LC-MS/MS) became the official method for monitoring diarrhetic shellfish poisoning (DSP) toxins as a result of uncertainties about the reliability of the mouse bioassay for detection of these toxins. As a result an almost ten year time series of toxin profiles in shellfish exists for some sites along the French coast, including Douarnenez Bay in Brittany (Fig. 1).

This shallow semi-enclosed bay, with an area of 230 km$^2$ (15 km wide by 20 km long) has a weak circulation which favours the accumulation of nutrients from different watersheds [2]. Water temperatures range between 7 and 21°C and salinity between 32 and 36 (minimum and maximum over 10 years). These features, combined with its particularly clear waters, favour phytoplankton growth in this area. This is particularly true from April to September when days are longer and sea surface temperatures increase. The Douarnenez coastline includes steep rocky seashore areas in the north and south and large sandy beaches to the east, with optimal conditions for clam (*Donax* spp.) cultivation (Fig.2 A-B).

Phytoplankton communities in Douarnenez Bay (Fig.1) have been monitored twice a month since 1987, as part of seafood safety and environmental quality control programmes. In parallel with phytoplankton monitoring, clams (*Donax* spp.) has been regularly analysed for lipophilic toxins before being marketed. These toxins include two groups of polyether compounds: i) diarrhetic shellfish poisoning toxins (DSP): okadaic acid (OA) and dinophysistoxins (DTXs: DTX1 and DTX2) and ii) pectenotoxins (PTXs: PTX1 and PTX2). These toxins are mainly produced by dinoflagellate species belonging to the genus *Dinophysis*.

During a typical year, the main phytoplankton species responsible for the occurrence of lipophilic toxins in Douarnenez Bay, from spring until late autumn or early winter, was *Dinophysis acuminata* (Figs.2C, 3A) [3]. In the last decade, densities of *D. acuminata* varied from 1 x 10$^2$ to 12 x 10$^3$ cells L$^{-1}$. The predominant toxin associated with this taxon was okadaic acid (OA) (Fig. 3 B). The maximum OA concentration

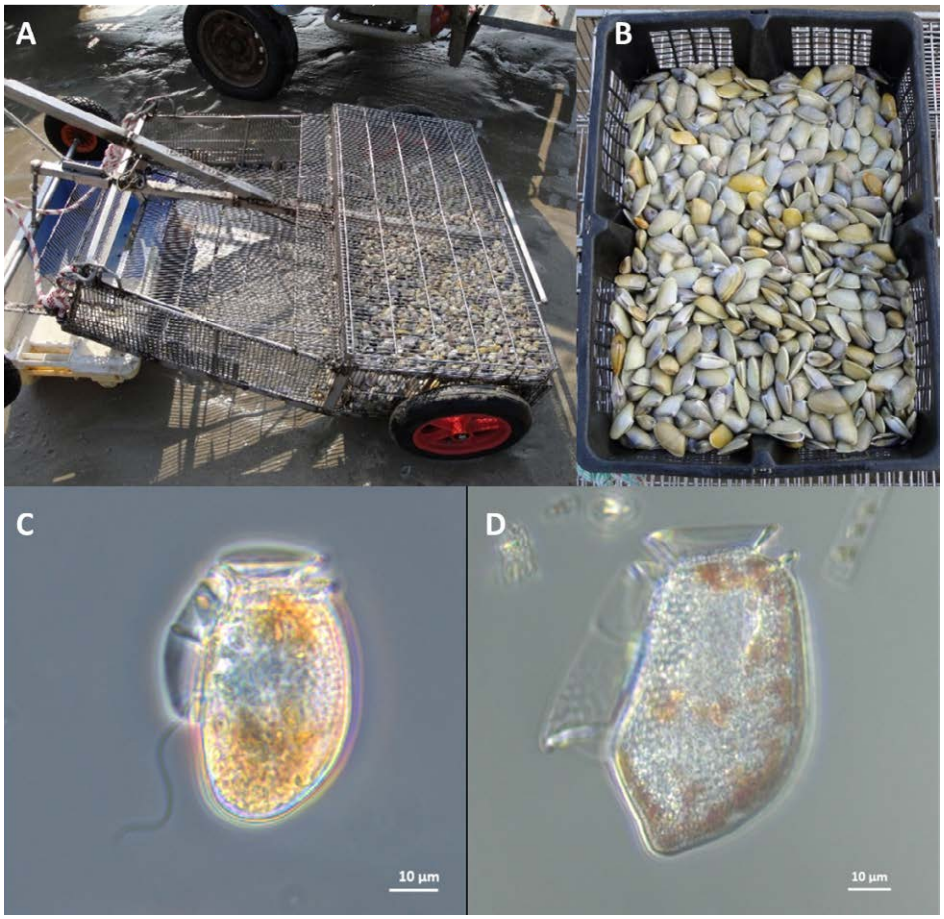


*Fig. 2. (A) Sampling device to collect (B) Donax clams. Light micrographs of (C) Dinophysis acuminata and (D) Dinophysis acuta. (Photos A, B by Dominique Le Gal; C, D by Audrey Duval).*

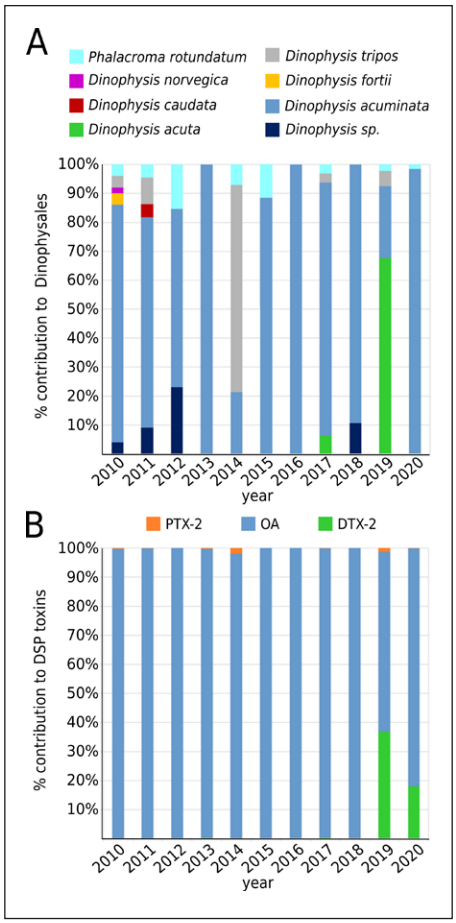


*Fig. 3. (A) Percentage of Dinophysis species in Douarnenez Bay water samples between 2010 and August 2020. (B) Mean percentage of DSP toxins in Douarnenez Bay in Donax spp. between 2010 and August 2020.*

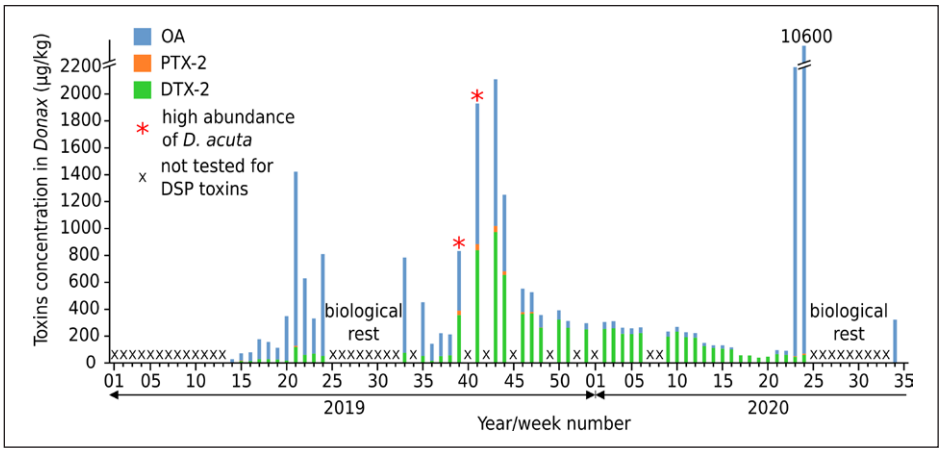


*Fig. 4. Weekly lipophilic toxin concentrations in Donax spp. in 2019 and 2020.*

recorded in *Donax* spp. was 9,853 µg $kg^{-1}$ in June 2020 (Fig. 4) [4]. The maximum toxin concentration as well as the duration of the episodes (concentration above the regulatory threshold) varied considerably from year to year. Usually, the other regulated DSP and PTX toxins were not detected (DTX1, PTX1) or were present at very low concentrations (DTX2, PTX2).

However, 2019 was a very exceptional year in terms of phytoplankton and toxin composition in Douarnenez Bay. Indeed, for the first time in over 30 years, *D. acuta* (Fig. 2D), known to produce OA and DTX2, was present in high cell densities in late summer and early autumn. Since 1987, *D. acuta* had never been observed in densities above $10^2$ cells $L^{-1}$ in Douarnenez Bay, but in September 2019, densities of 5.5 x $10^3$ cells $L^{-1}$ (week 39) and 3.3x $10^3$ cells $L^{-1}$ (week 41) were detected. After this bloom of *D. acuta*, *Donax* spp. were found for the first time to contain almost the same concentration of DTX2 as OA (Fig. 4). During week 39, when *D. acuta* was first detected (5.5 x $10^3$ cells $L^{-1}$), DTX2 and OA concentrations in *Donax* spp. were 356 µg $kg^{-1}$ and 443 µg $kg^{-1}$, respectively and in week 41, after the second detection of *D. acuta* (3.3x $10^3$ cells $L^{-1}$), 840 µg $kg^{-1}$ and 1,045 µg $kg^{-1}$ respectively. Finally, in week 43, maximum concentrations of DTX2 (974 µg $kg^{-1}$) and OA (1089 µg $kg^{-1}$) and low concentrations of PTX2 were recorded. Analysis of the full dataset from the REPHY monitoring network reveals that this is the first time such high DTX2 concentrations have been found in Douarnenez Bay. These are also the highest concentrations of DTX2 recorded in France to date.

The difference in depuration time for DTX2 and OA in *Donax* spp. is striking. While OA concentrations decreased very quickly, DTX2 was hardly eliminated at all (Fig. 4). During week 46, three weeks after the toxicity peak, DTX2 concentration was double that of OA, and still five times higher than that of OA eight weeks later (week 51). The presence of DTX2 at such concentrations and the difficulty of eliminating this toxin resulted in the regulatory threshold of 160 µg $kg^{-1}$ (OA+DTXs+PTXs) TEF being exceeded until early March 2020. Hence, the toxic episode of 2019 and first quarter of 2020 lasted a total of 49 weeks, including 27 weeks caused by the presence of DTX2 produced by *D. acuta*. Considering that in previous years *Donax* spp. harvesting bans due to DSP toxins in Douarnenez Bay lasted 10-29 weeks per year, the 2019 outbreak was an exceptionally long event. If blooms of *D. acuta* become recurrent, causing lengthy contamination by DTX2 in shellfish, a strong economic impact on *Donax* spp. harvesting activity is to be expected. Further investigations should aim at better understanding the reasons of this shift in the phytoplankton community.

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# New insights on the diversity of the dinoflagellate genus *Ostreopsis* in lagoons of French Polynesia, South Pacific Ocean

French Polynesia is a vast territory in the South Pacific Ocean, stretching over an expanse of more than 1,200 miles with a surface area as large as Europe. It is composed of 118 geographically dispersed islands and atolls regrouped into five distinct archipelagoes: Society Archipelago, Tuamotu Archipelago, Gambier Archipelago, Marquesas Archipelago, and Australes Archipelago (Fig. 1).

Historically, French Polynesia has long been concerned by harmful algae events, especially ciguatera poisoning (CP) which is, by far, the most prevalent seafood poisoning in the region [1]. Some areas like Gambier Islands undergo recurrent high toxicity CP outbreaks which became the focus of major research conducted in the late 70s by R. Bagnis, T. Yasumoto and Y. Fukuyo. Their pioneering work led to the formal identification of *Gambierdiscus* as the dinoflagellate responsible for CP [2-3]. Since this milestone discovery, several decades of research on ciguatera have been conducted in French Polynesia, which is the unique Pacific island territory with a permanent ciguatera research unit (Louis Malardé Institute, ILM) [1].

In addition to *Gambierdiscus* species, other benthic and potentially toxic dinoflagellates have been identified in benthic assemblages of French Polynesian ciguateric biotopes [4-5], including *Ostreopsis* and *Prorocentrum* species, but their potential harm has remained unstudied. In the past decades, *Ostreopsis* has become highly problematic in several temperate and subtropical areas, due to the formation of intense blooms associated with the production of toxic compounds analogous to palytoxin that have negative impacts on human health [6]. As the risk posed by *Ostreopsis* spp. proliferations in French Polynesia has never been assessed, investigations were undertaken as part of the research project TATOO to study the diversity and toxicity of *Ostreopsis* species in various French Polynesian lagoons. The present study was based on both field material collected between 2016-2019 from eight distinct islands (Fig. 1) and several clonal strains that are part of the Laboratory of Marine Biotoxins culture collection at the *Institut Louis Malardé* (Tahiti, French Polynesia), where cultures are deposited. Samples from islands of the five archipelagoes were obtained. Taxonomic identifications were carried out using microsocopy (LM, SEM) coupled with molecular characterization of DNA extracts prepared from cultures or single cells isolated from field samples. Toxicity screening analyses were initially conducted using the neuroblastoma cell-based assay (CBA-N2a), and toxin profiles were further characterized in toxic strains by liquid chromatography tandem mass spectrometry (LC-MS/MS) [7,9].

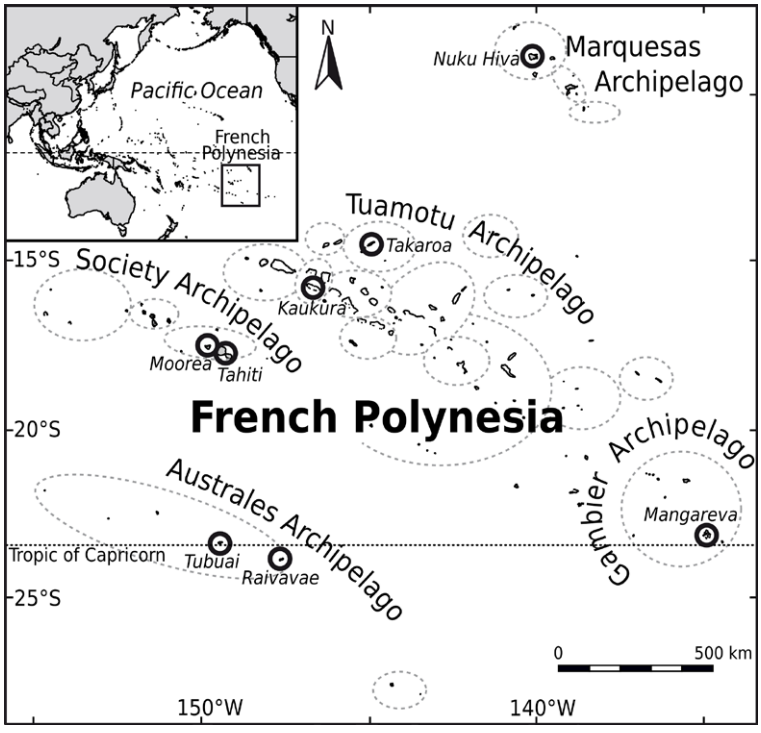


*Fig. 1. Map of French Polynesia showing the five archipelagoes. Islands where samples have been collected for this study are circled and their names are in italics.*

Our analyses revealed that two species, namely *Ostreopsis lenticularis* and *Ostreopsis* cf *ovata,* were present in all five archipelagoes of French Polynesia and that they constituted the most commonly observed *Ostreopsis* species in the area. This result is in agreement with previous observations by Bagnis and Fukuyo [4-5] and it was suggested that *Ostreopsis lenticularis* filled the ecological niche following *Gambierdiscus* outbreaks [5]. Thanks to our study, it was possible to re-investigate the type locality (Tahiti island) and unambiguously identify *Ostreopsis lenticularis*, confirming its morphological features and resolving its genetic identity (= *Ostreopsis* sp. 5) [7].

In addition to these two species, two other previously unreported species were found in several locations (Fig. 2).

In Kaukura and Takaora Islands (Tuamotu) as well as in Nuku Hiva Island (Marquesas) (Fig. 1), a small species (ca. 40–50 µm diameter) was present, and its thecal plate pattern was not significantly distinctive from other *Ostreopsis* species. Interestingly, it possessed a long second apical plate 2', reaching the fourth precingulary plate 4'' dorsally (Fig. 2B). Genetically, these specimens were identified as *Ostreopsis rhodesiae*, a species described rather recently from Heron Reef Lagoon, in the southern Great Barrier Reef (Coral Sea, Australia) [8] and, to our knowledge, not reported elsewhere to date.

An additional species was also observed in 2019 in Tahiti Island forming a large benthic bloom [9]. Morphologi-

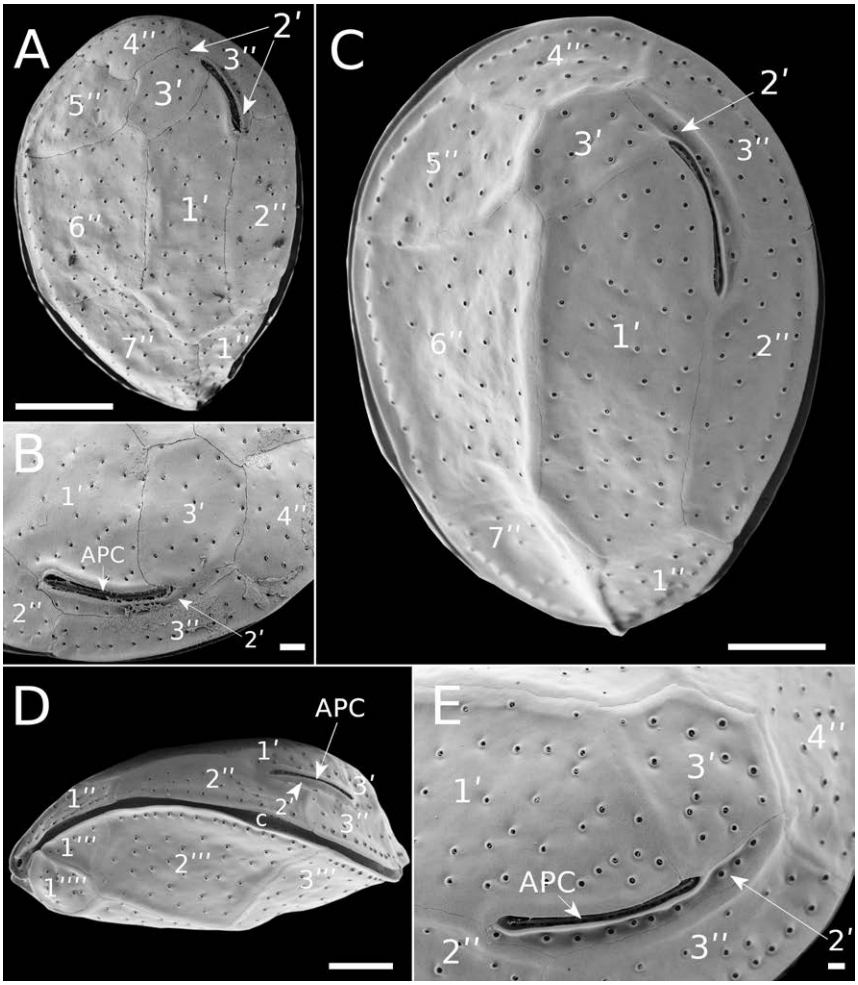


*Fig. 2. Scanning electron micrographs of two previously unreported Ostreopsis species in French Polynesia. (A) Apical view of O. rhodesiae. (B) Detail of the apical pore complex (APC) of O. rhodesiae. (C) Apical view of Ostreopsis sp. 6. (D) Left lateral view of Ostreopsis sp. 6 showing the undulated cingulum (c). (E) Detail of the APC of Ostreopsis sp. 6. Scale bars = 10 µm (A and C, D), 2 µm (B and E).*

cally, it was easily distinguished from other species by a typical undulation of the cingulum (Fig. 2D) and a rather large size (58-82 µm) [7]. Interestingly, this species is closely related morphologically to *O. siamensis*, as described by Schmidt [10] and interpreted by Fukuyo [4]. However, our detailed study revealed that it also has a long plate 2' (Fig. 2E), and this feature now appears to be common to several *Ostreopsis* species and probably not a relevant taxonomic character [9]. In this species, thecal pores were surrounded with a small collar rim, which was typical and absent in *O. rhodesiae* (Fig. 2). Genetic analysis revealed that this species corresponds to *Ostreopsis* sp. 6, which is also found in several tropical areas such as Gulf of Thailand, Malaysia, Viet Nam, Japan and more recently Korea [9]. Following this outbreak in Tahiti, this species was also observed in Moorea Island in November 2019, indicating that it is widespread in the Society archipelago. Both the genetic proximity of *Ostreopsis* sp. 6 sequences from Tahiti from those of the Gulf of Thailand, and the type locality of *O. siamensis* [10] suggest that it can be the same species. Fukuyo [4] emphasized the absence of *O. siamensis* in the samples from French Polynesia in the early 1980s, and it was not observed before 2019. Hence our observations constitute a new record of this species in the Pacific Ocean.

Preliminary toxicity analyses revealed that none of the *O. lenticularis* (× 47), *O.* cf. *ovata* (×13) and *O. rhodesiae* (× 1) clonal strains screened by CBA-N2a showed toxic activity, whereas strains of *Ostreopsis* sp. 6 (× 8) proved toxic, with a toxin profile dominated by ostreocin-D as confirmed by LC-MS/MS [9]. Due to the lack of analytical standards, its toxin profile is not completely resolved as yet, however, as *Ostreopsis* sp. 6 is capable of forming large blooms, these findings warrant further investigation on the potential environmental and/or health hazards posed by the proliferation of this species in French Polynesian lagoons. Future studies should aim at developing a better understanding the biogeographic distribution of this species, as well as assessing the impacts of its associated toxins on coral reef ecosystems and/or putative accumulation in marine organisms.


## Acknowledgements

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# Toxin profiles of *Gambierdiscus lapillus* from the Cook Islands

Species of the dinoflagellate genus *Gambierdiscus* produce the toxins responsible for ciguatera fish poisoning (CFP), an illness that has been prevalent throughout the Pacific and particularly in the Cook Islands [1]. The illness is caused by ciguatoxins (CTXs) and possibly maitotoxins (MTXs). Although CTX production ability is genetically associated with the *Gambierdiscus / Fukuyoa* complex, the presence of *Gambierdiscus / Fukuyoa* in a particular area does not mean a risk of CFP, as the toxicity of the species and even strains of species can vary [2]. The key species known to be responsible for CFP in the Pacific is *G. polynesiensis*, but other species need to be considered and their toxin profiles determined to enable better risk assessments. This is particularly important for regions where illnesses have been reported but *G. polynesiensis* has not been detected.

The epiphytic species *Gambierdiscus lapillus* was first isolated from Heron Island, Queensland, Australia, where CFP is endemic [3]. Culture extracts of these Queensland isolates were tested by mouse biaoassay (MBA) and were toxic to mice, however liquid chromatography with tandem mass spectrometry (LC-MS/MS) analyses of these isolates were negative for the known microalgal Pacific ciguatoxins (P-CTXs; P-CTX-3B and C, P-CTX-4A and B) and maitotoxin-1 (MTX-1). They did, however, produce 44-methylgambierone (44-MG) [3-5] although this compound is not considered a cause of CFP [6]. Trace-level toxin activity was also detected using the $Ca^{2+}$ influx SH-SY5Y cell Fluorescent Imaging Plate Reader bioassay. It was hypothesised that unknown compounds were responsible for the MBA results and that *G. lapillus* could still be a contributor to CFP intoxications in Australia [3].

*Fig. 1. Sampling area in Rarotonga, Cook Islands.*

*Fig. 2. Typical macroalgal substrate, Halimeda species.*

Sampling of macroalgae was carried out in Rarotonga, Cook Islands, at Muri Lagoon in March 2017 and at Titikaveka Beach and Muri Beach (Fig. 1) by Prof Muharrem Balci in August 2019. Samples were returned to the Cawthron Institute, New Zealand, under quarantine regulations and living cells were immediately isolated and on-grown for molecular and toxin analyses. Species determination was by DNA sequencing (large subunit ribosomal DNA, D8-D10 region) as described previously [7]. Production of P-CTXs [8], MTX-1 [9] and 44-MG [4] by isolates was determined by LC-MS/MS. Isolates were deposited in the Cawthron Institute Culture Collection of Micro-algae (CICCM).

Two isolates from samples collected in 2017 (CAWD263 and 264) and six isolates from the 2019 sampling effort were confirmed as *G. lapillus* (CAWD330-333, 335, 338) (Fig. 2). All were positive for 44-MG production and negative for microalgal P-CTXs and MTX-1 production.

Other *Gambierdiscus* species isolated from the 2017 event were *G. polynesiensis* (CAWD267) and *G. pacificus* and in 2019 *G. pacificus* (CAWD337) (Fig. 3). Only the *G. polynesiensis* isolate produced the known microalgal P-CTXs [unpublished data], while for both *G. polynesiensis* and *G. pacificus* 44-MG was detected. Other *Gambierdiscus* species isolated from the Cook Islands from earlier sampling efforts were *G. australes, G. cheloniae* and *G. honu.* Only *G. australes* produced MTX-1 but all produced 44-MG [10].

The use of molecular assays to determining the presence of *Gambierdiscus* species in field samples is proving useful for guiding isolations [e.g., 11-13]. As *G. lapillus* has been considered a potential candidate for CFP [3,14], a species-specific quantitative PCR assay for *G. lapil-*

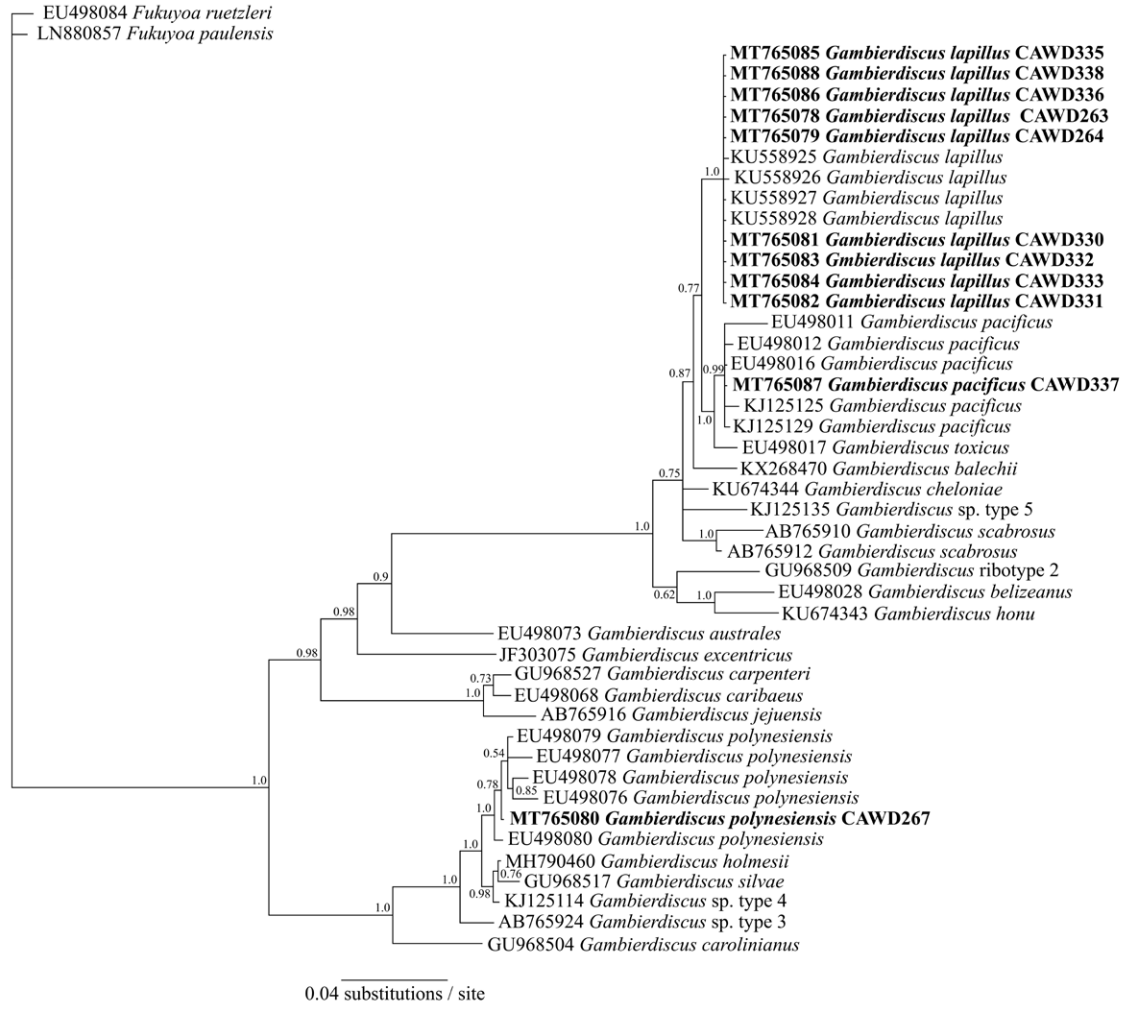


*Fig. 3. Phylogenetic analysis of partial large subunit ribosomal DNA sequences (D8–D10 region) from the Gambierdiscus strains isolated in this study (in bold font) using Bayesian analyses. Values at nodes represent Bayesian posterior probability support. Scale bar is substitutions per site.*

*lus* was developed and has proved sensitive and accurate [14]. Molecular tools will be used to analyse future samples collected not only from the Cook Islands but from throughout the Pacific region including Aotearoa/New Zealand and its territories (Rangitāhua/Kermadec Islands) where, due to climate change, CFP is considered an emerging risk.

Based on the current results, it is unlikely that *G. lapillus* is a primary cause of CFP, but further toxicity work is planned to determine whether it does produce currently undescribed compounds which could contribute to CFP cases.


## Acknowledgements

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*Assoc Prof Muharrem Balci on sabbatical at Cawthron Institute, Nelson, New Zealand, 2019*

# Unusual bloom of the red alga *Ceramium* sp. (Ceramiales, Rhodophyta) in Cartagena, Colombia, SW Caribbean Sea

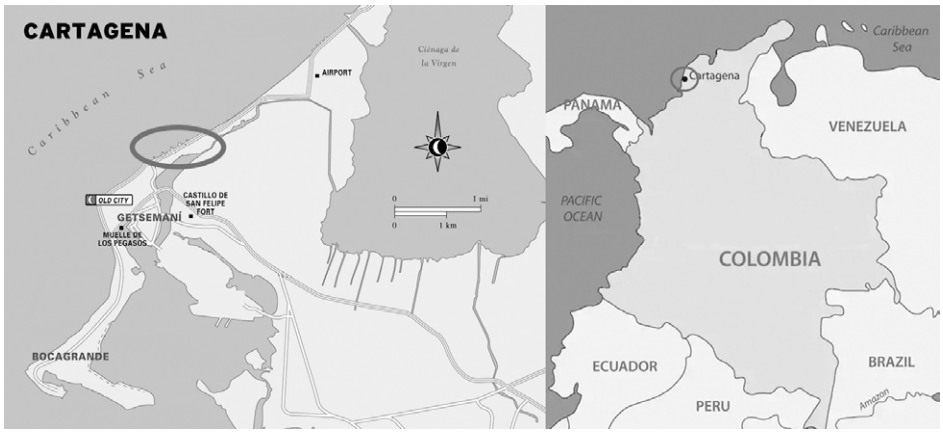


*Fig. 1. Map of the study site.*

Macroalgal blooms are frequently associated with eutrophication of coastal waters [1]. These blooms are mainly composed of ephemeral and opportunistic green algae belonging to the genera *Ulva*, *Chaetomorpha* and *Cladophora*. Less frequently, red algal species are involved, and when this occurs, the genera *Hypnea* and *Gracilaria* are usually involved [2-4]. Macroalgal blooms have been increasing in frequency, geographic distribution and duration in the last four decades [4]. Unlike toxic phytoplankton blooms, macroalgal blooms lack chemical toxicity but have other ecological impacts. They may displace indigenous species, alter biogeochemical cycles, increase grazing and cause die-off of seagrass and coral reefs [5-6]. Furthermore, when drift algae reach the coastline they foul beaches and shorelines important to local tourist economies and require expensive biomass removal programs [6-7].

Cartagena de Indias is one of the main tourist attractions in Colombia. In addition to the historical town center and its architecture, tourists are attracted by its beaches. Since the 1980s there are records of macroalgal blooms in Marbella beach, at the north end of town [8]. The authors reported a mixed bloom occurring in 1988, dominated by the red algae *Gracilaria cylindrica*, *Hypnea musciformis* and *Grateloupia filicina*. During September-October of the last two years (2018 and 2019), a monospecific red algal bloom has been observed in the same locality in Marbella beach (Figs 1-2). The taxon involved is an unidentified species of *Ceramium* (Fig. 3). Its morphological features do not correspond to any of the listed taxa for the Caribbean Sea, and it was not possible to identify it to species level. During 2019, several species of dead animals were observed, entangled by the wrack biomass of *Ceramium* (Figs 4-5). Species of *Ceramium* are not re-

*Fig. 2. Aerial view of the bloom. Photo courtesy of Luz Marina Mejia.*

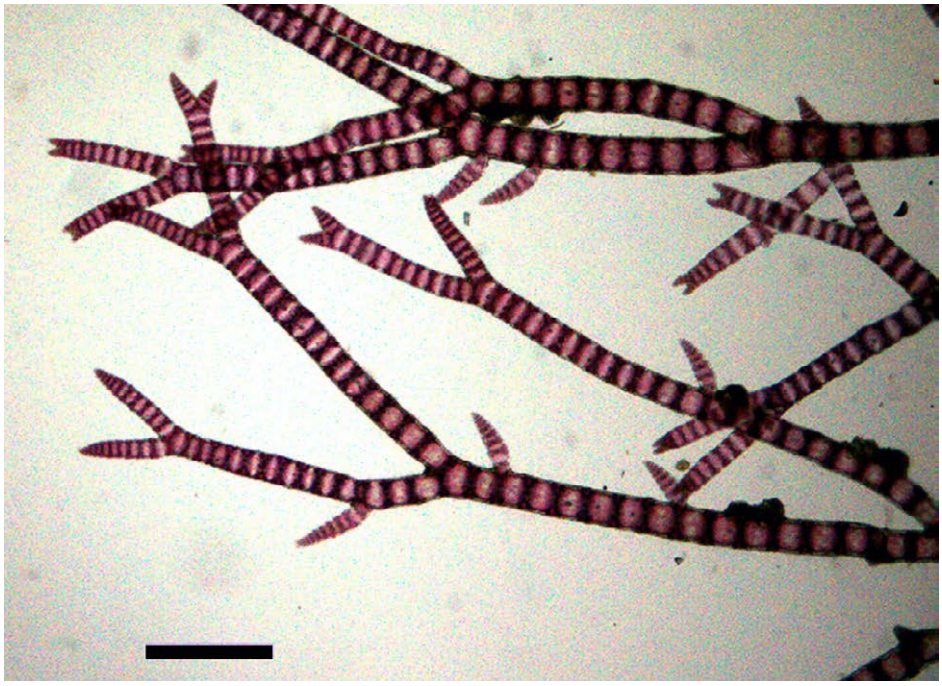

*Fig. 3. Ceramium sp. Scale bar: 200µm.*

*Fig. 4. Dead jellyfish entangled by Ceramium stranded on the beach.*

*Fig. 5. Dead fish recovered from wrack Ceramium.*

ported as bloom forming species, and this finding adds another genus to the group of harmful bloom-forming macroalgae. Furthermore, this report highlights the potential introduction of a new species which has passed undetected until now. This would not be the first case of a potentially introduced bloom species: an undescribed species of the genus *Anadyomene* was reported forming a persistent bloom in nutrient enriched waters in Florida [9].

The invasive species *Caulerpa brachypus* f. *parvifolia* (Harvey) A.B. Cribb has bloomed in Florida as well [3]. Molecular analyses needs to be carried out in order to assess the identity of this bloom-forming species of *Ceramium*.

## Acknowledgments

The author thanks Dr. Carlos Camargo-Bareño for informing her about the bloom. Prof. Luz Marina Mejia, Universidad del Sinú-Cartagena, provided the aerial photographs. Field sampling was partially financed by the Facultad de Ciencias and Vicerrectoria de sede Bogotà, Universidad Nacional de Colombia.

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# Distribution of the fish-killing dinoflagellate *Karlodinium* (Dinophyceae) in the Johor Strait, Malaysia

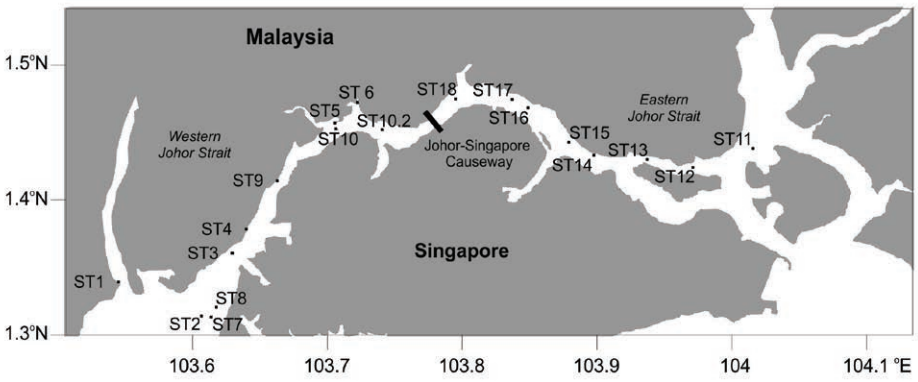


*Fig. 1. Sampling sites in the Johor Strait*

Species of *Karlodinium* are naked dinoflagellates. More than one third of the named species have been known to cause fish mortality. Toxigenic *Karlodinium* species have gained notoriety due to their capability of producing ichthyotoxins (karlotoxins) that damage the fish gill epithelial tissues causing mortalities by suffocation [1]. In this study, the spatial and temporal distribution of the dinoflagellate *Karlodinium* in the Johor Strait was investigated. The strait is a narrow waterway that separates Peninsular Malaysia and Singapore (Fig. 1) and is an important aquaculture area for both countries. Blooms of *Karlodinium australe* previously reported in the western Johor Straits have led to massive caged-fish mortality and serious economic losses in 2014 and 2015 [2-4].

A total of 19 stations along the Johor Strait were sampled during plankton monitoring surveys between January 2018 and September 2019 (Fig. 1). *Karlodinium* species were present in the Strait but at low cell densities ranging from $2 \times 10^2$ to $5.5 \times 10^3$ cells $L^{-1}$ (Fig. 2). The average cell density of *K. australe* recorded during the 2014 bloom was $1.25 \times 10^6$ cells $L^{-1}$, with a maximum ($2.34 \times 10^6$ cells $L^{-1}$) recorded at the innermost part of the Strait [2]. Densities of *Karlodinium* exceeding 5,000 cells $L^{-1}$ were observed in October 2017 (S18), February–April 2019 (S4, S17, and S18), and July 2019 (S14). Results from a Spearman's rank correlation revealed that phosphate ($PO_4$-P) availability in the Strait was significantly correlated with high abundances of *Karlodinium* ($p < 0.05$). It has been suggested that the 2014 bloom was probably triggered by high nutrients input from the nearby river discharges and anthropogenic inputs from mariculture, coastal reclamation and dredging activities in the Strait [2]. The stagnant water conditions during neap tides also contributed to sustain the bloom [2]. This view is supported by Leong et al [5] who showed that dinoflagellates blooms including *K.* cf. *veneficum* and *K. australe* in the Eastern Johor Strait occurred during neap tides with high nitrogen but low silicate concentrations. Diatoms were likely suppressed under silicate depletion, allowing the dinoflagellates to thrive in that environment, and subsequently prolong their bloom development in the Strait [5-6].

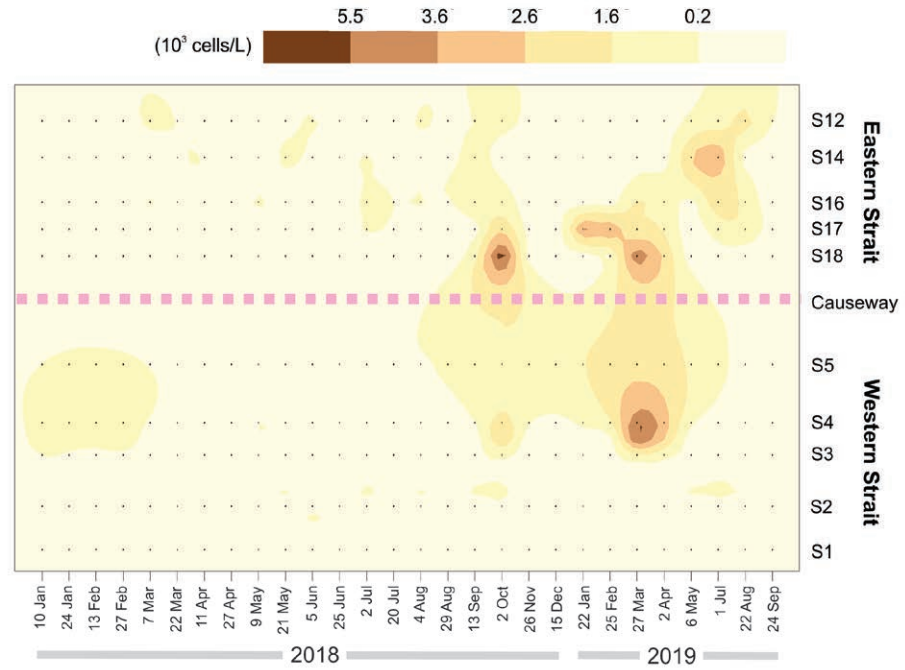


*Fig. 2. Spatial and temporal distribution of Karlodinium along the Johor Strait during the study period between January 2018 and September 2019*

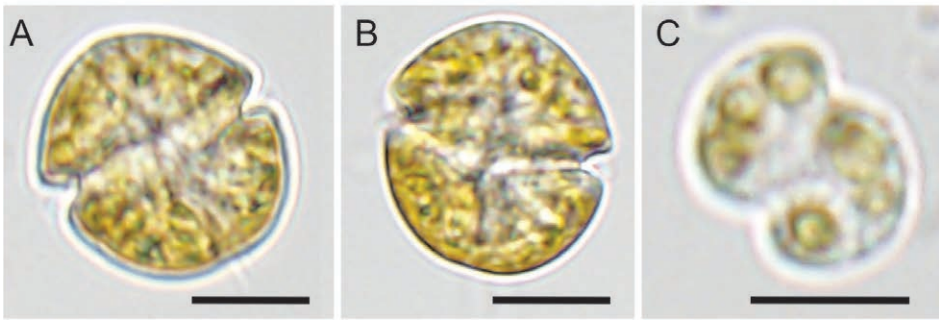


*Fig. 3. Light micrographs of Karlodinium found in the Johor Strait. Scale bar = 20 µm.*

Under the light microscope (LM), cells of *Karlodinium* appear oval with epicone and hypocone equal in size. The shape of the epicone is conical or rounded while the hypocone is hemispherically rounded (Fig. 3A) and the

*Continued on page 16*

# Bloom of a red tide species *Akashiwo sanguinea* in Semerak Lagoon, Kelantan, Malaysia

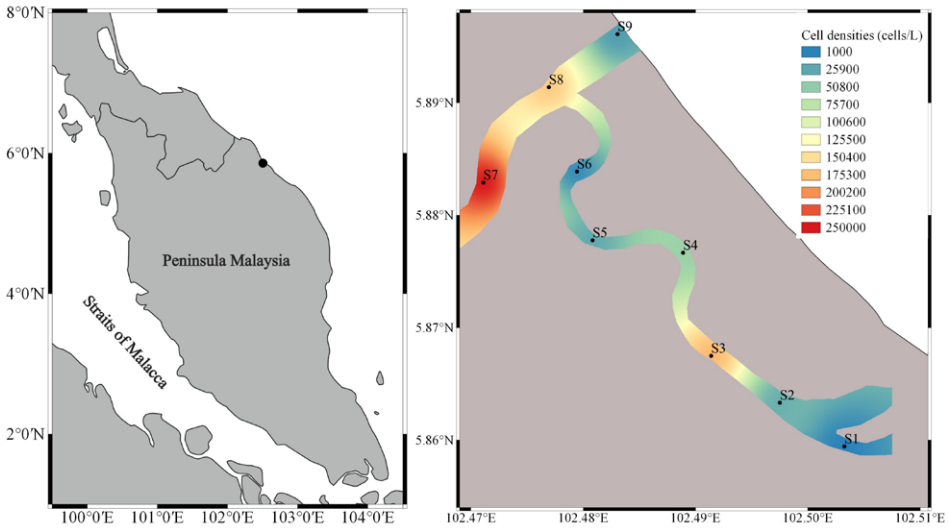


*Fig. 1. Chart of sampling sites at the Semerak Lagoon and cell densities of Akashiwo sanguinea*

The species *Akashiwo sanguinea* is a cosmopolitan naked dinoflagellate known to form harmful algal blooms (HABs). This bloom-forming species has been reported to cause seabird mortality due to saponification in Monterey Bay, California, USA [1]. In Malaysia, there are no reports of fish kill incidents caused by *A. sanguinea* to date, but the species is commonly found in Malaysian waters, especially in aquaculture areas [2-3].

The Semerak Lagoon, located in the north east of Peninsular Malaysia, is an important aquaculture area for finfish and shrimp. On May 23th, 2019, water discoloration was observed in the lagoon (Fig. 1). Following the incident, morphological and molecular analyses were performed to identify the bloom species. Water samples from nine selected sampling sites (S1–S9; Fig. 1) were collected at 1 m depth using an 8-L Van Dorn water sampler. The sampling stations S1 to S3 were located inside, S4 to S8 outside the aquaculture area, and S9 at the river mouth. Clonal cultures were established through single-cell isolation from field samples. Field and cultured cells were observed using light microscopy and confirmed the species as *Akashiwo sanguinea*. Cells were 60.6 to 81.0 µm long and 46.6 to 60.6 µm wide with numerous ribbon-like chloroplasts in the periphery (Fig. 2).

Gene amplification of the large subunit (LSU) region and the internal transcribed spacer (ITS) region was performed by polymerase chain reaction. The species was previously reported to comprise four ribotypes (A, B, C, and D) [4]. Our results based on both LSU rDNA and ITS sequence analysis clearly indicated that the *A. sanguinea* population in Semerak Lagoon belonged to ribotype B; sharing the same ribotype with the strains found in Tumpat, Kelantan [4]. The LSU phylogenetic inference revealed that the strains from Semerak Lagoon shared identical sequences with those from China, Mexico, Singapore, and South Korea (Fig. 3A; MP/ML/BI: 99/99/1.0); while using ITS phylogeny, all strains from Semerak Lagoon shared identical sequences with one strain (GSXM02) from Xiamen Harbour, China (Fig. 3B; MP/ML/BI: 100/100/0.99).

During the bloom event, the highest density of *A. sanguinea* was found at S7 (248,658 cells $L^{-1}$; outside the aquaculture area), followed by S3 (177,773 cells $L^{-1}$; inside the aquaculture area), and S8 (164,269 cells $L^{-1}$; outside aquaculture area) (Fig. 1); the densities were higher as compared to the previous occurrences in 2015 and 2016 (highest density of 3,460 cells $L^{-1}$; [3]). The phytoplankton community assemblage in the lagoon was diverse, with a dynamic shifting of phytoplankton community composition over time related to the nutrient dynamics in the lagoon. Aside from blooms of *A. sanguinea*, several different algal blooms were encountered between the study period of September 2015 and March 2016: i.e. *Pseudo-nitzschia* spp. (potentially toxic species), *Chaetoceros*, *Skeletonema*, and *Blixaea quinquecornis* (red tides, fish kills) [3].

In this survey, we confirmed the blooming species as the dinoflagellate *A. sanguinea* belonging to ribotype B. The potential occurrence and distribution of other ribotypes require further investigation, and the drivers of the recurrence of this species remain unknown. Therefore, routine monitoring along the lagoon is needed for early warning to minimize losses due to fish kill events.


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*Fig. 2. Light micrograph of Akashiwo sanguinea from Semerak Lagoon. Scale, 20 µm*

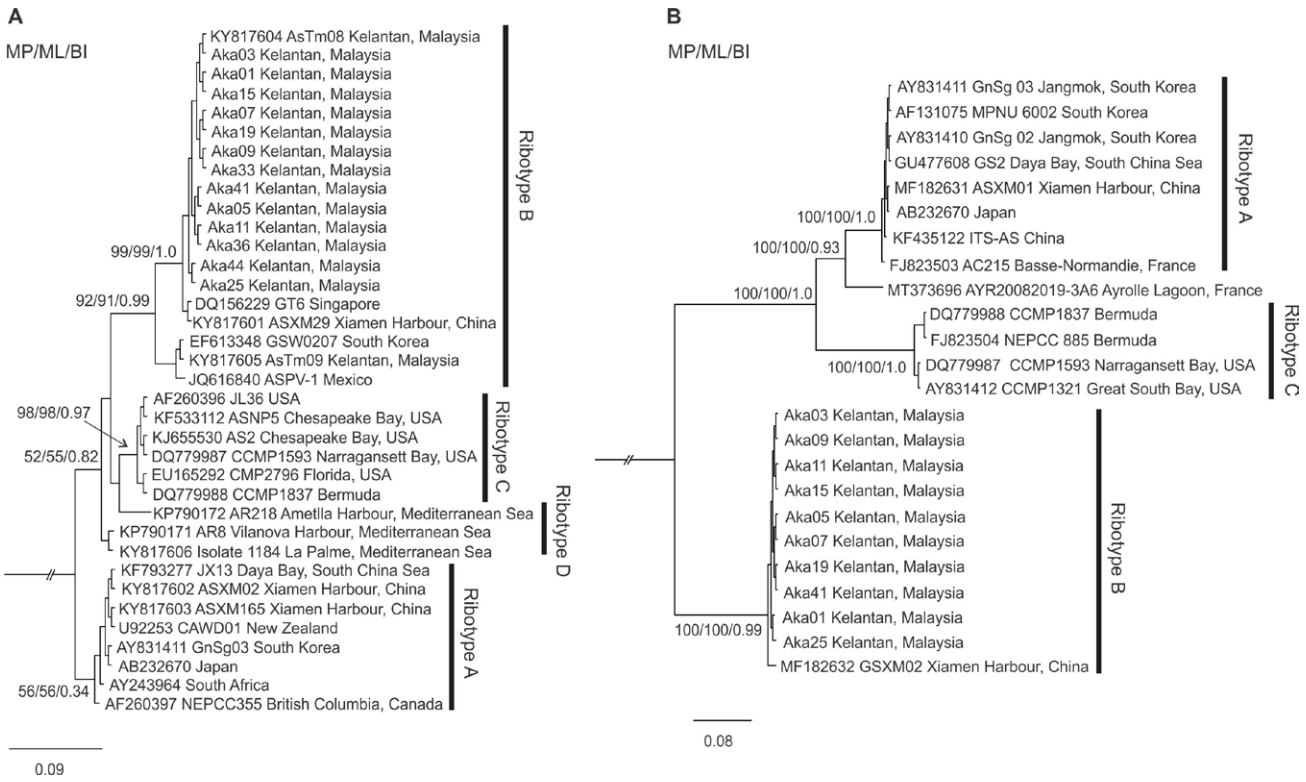


*Fig. 3 Bayesian trees of Akashiwo sanguinea inferred from (A) LSU rDNA and (B) ITS datasets. Values on nodes represent bootstrap supports of MP, ML, and posterior probabilities of BI*

*Continued from page 14*

sulcus extension invading the epicone is visible (Fig. 3B). Cells are slightly pigmented, with chloroplasts observed along the epicone and hypocone, each with a lenticular pyrenoid (Fig. 3C). Observations of *Karlodinium* cells collected during the sampling period were 10.2 – 17.4 µm long (11.8 ± 1.2 µm, $n$ = 10) and 8.4 – 13.6 µm wide (8.9 ± 1.1 µm, $n$ = 10), with a length: width ratio of 1.60 ± 0.2 µm. Thus, their size was slightly smaller than those described in [2] and [7]. However, with the data obtained here, it was not possible to identify the specimens at species level.

To gain a better insight into the species distribution and diversity, the assemblages of *Karlodinium* in Johor Strait were characterized with a genetic approach using ribosomal DNA (rDNA) metabarcoding and real-time quantitative PCR (qPCR). Plankton samples collected between May 2018 and September 2019 were further analysed with the 18S-V9 rDNA metabarcoding approach using a high throughput sequencing under the Illumina Miseq platform. Our results revealed a total of 27 operational taxonomic units (OTUs) associated with harmful microalgal species. Among the harmful algal OTUs, *K. veneficum* was detected at the Eastern Johor Strait (S14 and S16) during the Northeast- (January 2019) and Southwest (July-August 2019) monsoons but in relatively low (number of OTU) abundance. Results from a Pearson correlation showed that nitrate concentration in the strait were significantly associated with OTU abundances of *K. veneficum* ($p < 0.05$). Nevertheless, *K. australe*, the previously reported fish-killing species, was not detected in our metabarcoding datasets during the study period. Likewise, the qPCR species-specific detection of *K. australe* [8] confirmed no detection of this species during the study period.

Clearly, further monitoring efforts are warranted to alleviate the adverse impacts of HABs on the aquaculture industry in the Strait of Johor.

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# CLEFSA project identifies Harmful Algal Blooms as a threat to food safety resulting from climate change


*Fig. 1. Organizations involved in the CLEFSA project*

Climate change is one of the key drivers of emerging risks for food and feed safety, plant and animal health (including terrestrial and aquatic species) and food nutritional quality. In order to ensure food and feed safety in the future, analysis of environmental drivers may contribute to supporting long-term anticipation of food safety challenges and risk assessment needs, as well as the design of prevention measures.

The CLEFSA project (Climate change and Emerging risks for Food Safety) has developed and tested new methodologies for the identification, characterisation and analysis of a large number and variety of emerging risks linked with climate change, including those associated with marine toxins. European Union and United Nations (e.g. UNESCO-IOC) agencies, international organisations and coordinators of relevant international projects and programmes including some directly related to harmful algal blooms (EuroCigua project, GlobalHAB programme) were engaged to build a knowledge network of experts (Fig. 1). The overall CLEFSA procedure is illustrated in Fig. 2.

Horizon scanning and crowdsourcing were used to collect a broad range of signals or emerging issues from a variety of information sources. CLEFSA has characterised them through a Multi-Criteria Decision Analysis (MCDA) tool (Fig. 3).

Characterisation has been conducted in a participatory manner through the involvement of a multidisciplinary group of 60 experts, who have assessed how climate change affects the impact and likelihood of emergence of issues. Climate change scenarios, using the climate data store provided by the Copernicus C3S platform implemented by the European Centre for Medium-Range Weather Forecasts (ECMWF), were used.

The project has developed methodologies and indicators for the analysis and visualisation of the information collected during the characterisation, as well as for addressing the uncertainty associated with these assessments.

Rather than comprehensively describing all issues individually, the CLEFSA project has focused on the multifaceted effects of climate change, the diversity of hazards to be considered, the large uncertainties involved and the interconnections between the different issues.

Over 100 emerging issues were identified and characterised, among

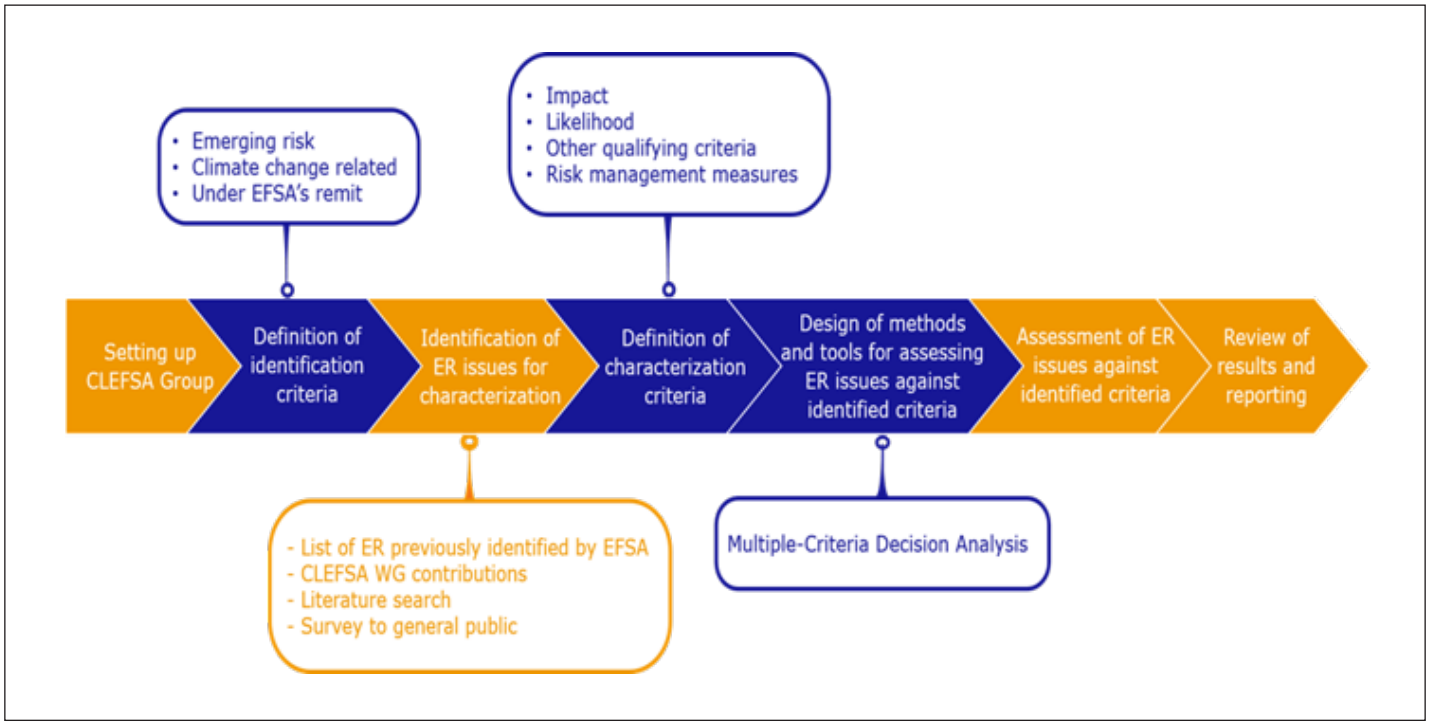


*Fig. 2. Chart diagram of CLEFSA protocols*

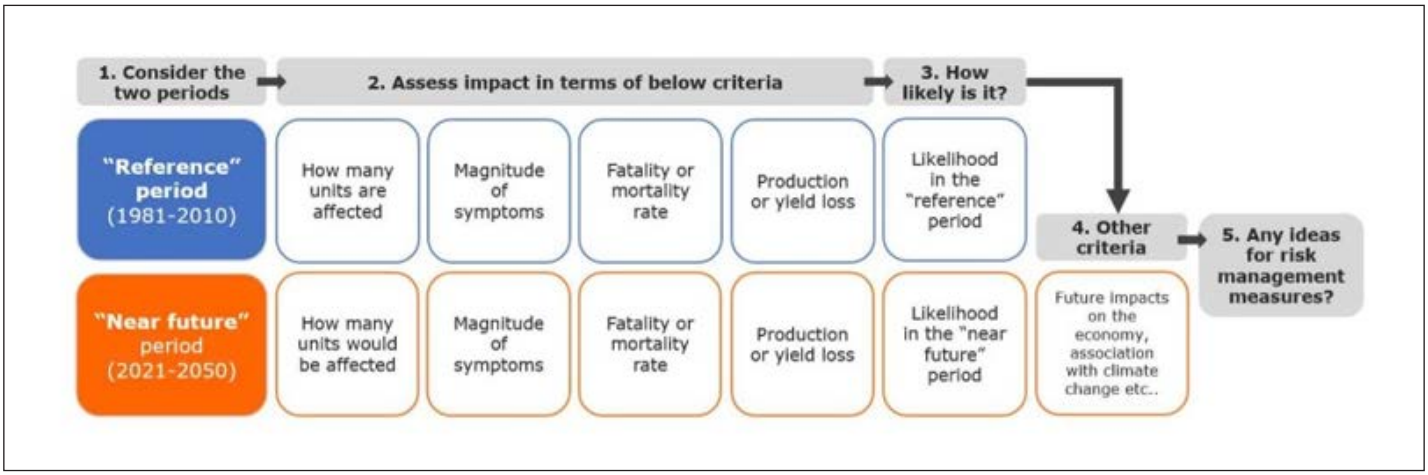


*Fig. 3. CLEFSA Multi-Criteria strategy*

which several directly related to toxins produced by harmful algal blooms (HABs). These include: ciguatoxin, domoic acid, okadaic acid, saxitoxin, pinnatoxin, tetrodotoxin, beta-methyl-amino-L-alanine (BMAA) and palytoxin analogues. The analysis indicates that climate change may increase severity, duration and/or frequency of the potential effects of these hazards. However, a more pronounced effect is expected on the likelihood of emergence. The analysis showed that issues related to HABs were also among the ones with the highest likelihood of emergence in the climate change scenario. Scientific literature and previous EFSA work (e.g. EuroCigua project) [1] indicate that climate change may be influencing the occurrence and intensity of blooms of potentially toxic marine algae and bacteria resulting in risk for food and feed safety.

The CLEFSA project has stressed the need for policymakers and other relevant actors in the food system to consider adjusting surveillance and monitoring to prepare for emerging risks to food and feed safety linked to climate change, and for risk assessors to consistently include climate change scenarios in risk assessments. Considering the increasing demand for new sources of food and feed from the ocean, the CLEFSA project contributes to raising awareness on the need of integrated policies for ocean protection and food safety by building synergies across the relevant institutional actors involved. The complete CLEFSA report can be found at *https://www.efsa.europa.eu/it/supporting/pub/en-1881*.

## Acknowledgements

EFSA wishes to thank the following experts for the support provided to this scientific output: Stefano Messori (OIE), Andrea Toreti (JRC), Hans-Martin Füssel (EEA), Elisa Berdalet (UNESCO-IOC and SCOR GlobalHAB programme), Jan C. Semenza (ECDC), Jessika Giraldi (JRC), Jorge Diogène (EuroCigua project), Cristina Tirado (University California Los Angeles), Marco Bindi (Florence University), Kim Petersen (WHO), Keya Mukherjee (FAO), Federica Rossi (CNR), Pinya Sarasas (UNEP), Robert Stefansky (WMO). Thanks also to the following EFSA Staff members: Angelo Maggiore, Ana Afonso, Federica Barrucci, Giacomo de Sanctis, Aleksandra Lewandowska, Angeliki Konsta, Yves Van der Stede, José Tarazona; to all the experts listed in Appendix A of the CLEFSA report as well as the following members of the EFSA Uncertainty Working Group: Andrew Hart, Ullrika Sahlin, Wopke van der Werf and Peter Craig.

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# Blooming Buddies: MSc Research Projects Extend our Knowledge on Bloom-Forming Freshwater Cyanobacteria

Freshwater cyanobacteria blooms are an increasing problem globally and much work is focussing on understanding bloom dynamics and toxin production in order to better manage the inherent health risks they pose. In New Zealand, the major toxin-producing cyanobacteria are microcystin-producing *Microcystis* and anatoxin-producing *Microcoleus* (previously classified as *Phormidium*) which frequently form blooms in New Zealand lakes and rivers, respectively. These cyanobacterial blooms have led to numerous animal deaths (dogs, sheep and cattle) and the closure of recreational swimming sites due to the human health risk at certain times of the year. In New Zealand, there has been a good deal of research conducted on microcystins which was recently reviewed in the *New Zealand Journal of Botany* [1]. This review revealed interesting observations about microcystin-production in New Zealand *Microcystis*; that there are predominantly two microcystin congener profiles produced by New Zealand *Microcystis*, and that there are geographical differences in microcystin quotas between international data and New Zealand *Microcystis* strains. It also identified that New Zealand research on microcystin production has contributed significantly to international knowledge on the topic.

In 2017, funding from the Marsden Fund of the Royal Society of New Zealand was awarded to investigate cyanobacterial bloom ecology and whether cooperation occurs between toxic and non-toxic strains of cyanobacteria. The premise for the project was that microcystins were sequestered by the non-toxic cyanobacterium *Synechocystis* and modulated its photosynthetic performance [2]. The project also included funding for two MSc scholarships that were awarded to Jenna Mumford and Rossella Nicolai. Jenna's research project centred around assessing the impacts of environmental contaminants on cyanobacteria (microcystins themselves and herbicides) and how this might affect the community composition. Rossella's work was on the potential active export of microcystins and the optimisation of a cell lysis staining technique for use with cyanobacteria. Both are enrolled through Victoria University of Wellington, but undertook their research projects at the Cawthron Institute in Nelson (New Zealand).

Jenna, from Wellington, has a passion for environmental sustainability. As such, she wished to investigate how global change is affecting aquatic environments and chose to investigate the effects of herbicides (glyphosate and 4-chloro-2-methylphenoxy acetic acid; MCPA) on New Zealand cyanobacteria. For her experiments, she used a selection of planktonic cyanobacteria from the Cawthron Institute Culture Collection of Microalgae [3] (Fig. 1) spanning seven species from four genera; including *Cuspidothrix issatschenkoi*, *Dolichospermum circinale*, *Microcystis aeruginosa* and *Nodularia spumigena*. During screening experiments, Jenna found that the cyanobacteria species assessed were not affected by MCPA (up to $5 \times 10^3$ µg $L^{-1}$) but they did show varied susceptibility to glyphosate. In a real-world situation, the differential effects observed with glyphosate might disturb the balance of cyanobacterial communities in lakes and reservoirs, and could potentially modify proliferation of toxin-producing species. Follow-up experiments are now being conducted to investigate the effect of glyphosate photosynthetic performance and toxin production in a selection of the susceptible cyanobacteria species.

Rossella hails from Rome, Italy, but has undertaken her tertiary education at Victoria University in Wellington (New Zealand). Rossella's passion for art has led her to develop an interest in algal microscopy during her university education, finding beauty in their cellular structure. Rossella's work on the potential active export of microcystins employed the fluorescent nucleic acid stain, SYTOX™ green [4-5], to measure the amount of cell lysis observed during culturing experiments using cyanobacteria. The SYTOX green stain is particularly useful for analysing cyanobacteria (and other microalgae) because its fluorescence characteristics (excitation 504 nm; emission 523 nm) avoid interference from chlorophyll autofluorescence (excitation 440/660 nm; emission 700 nm). During her project Rossella was able to optimise staining procedures for *Microcystis aeruginosa*, *Nodularia spumigena* and *Planktothrix* sp. (Fig. 2). However, the presence of heterotrophic bacteria in the non-axenic cyanobacteria cultures did prove troublesome in *Nodularia spumigena* samples, as heterotrophic bacteria were too abundant in the mucilage produced by the cyano-

*Fig. 1. Microcystis aeruginosa CAWBG11, one of the strains used by Jenna to assess the effects of herbicides on cyanobacteria.*

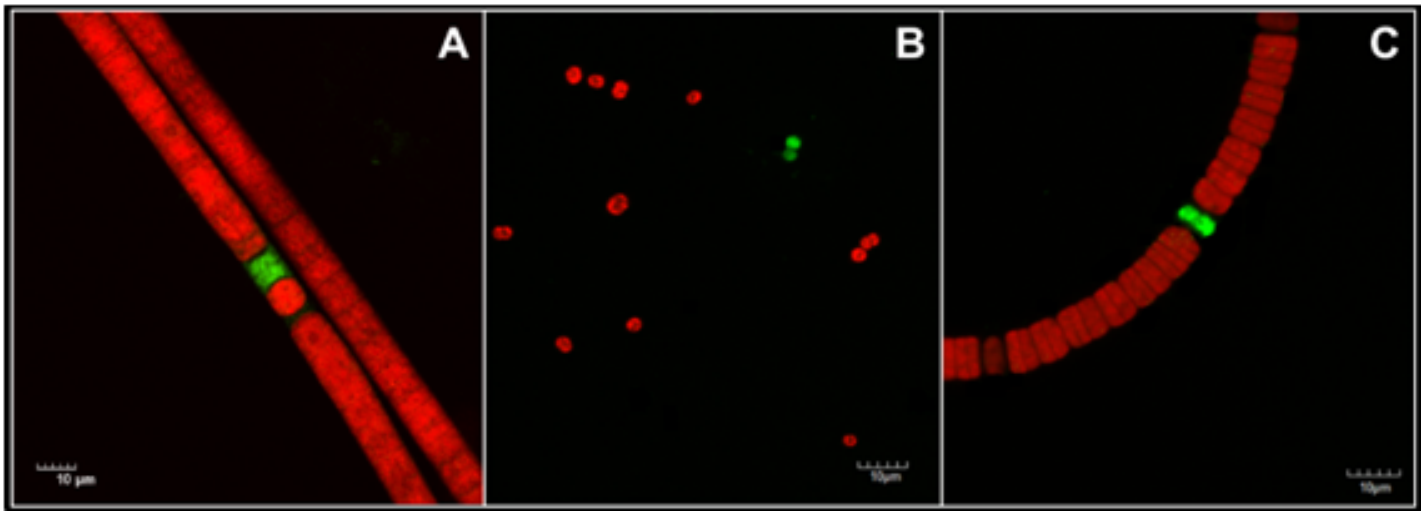


*Fig. 2. Confocal microscopy images of Planktothrix sp. CAWBG59 (A), Microcystis aeruginosa CAWBG617 (B) and Nodularia spumigena CAWBG21 (C) stained with SYTOX™ green so that lysed cells fluoresce green, whilst intact cells are detected by red chlorophyll autofluorescence.*

bacterium interfering with the cyanobacterial signal. Rossella is now using this fluorescence microscopy technique to assess whether cell lysis or active transport is the main contributor to the extracellular microcystins observed in cyanobacteria cultures. Because data on cell lysis has been lacking in previous experiments performed on microcystin export, the SYTOX green staining technique has proven valuable for our work in this area.

As Cawthron operates outside of the New Zealand University system and is largely staffed by scientists, technicians and consultants conducting government-funded and contract research, it's always refreshing to have the added vibrancy and enthusiasm of research students being based onsite. This latest collaboration with Victoria University of Wellington builds on previous collaborations working on toxic benthic cyanobacteria in rivers and will further cement the relationship between the two organisations.

*Rossella Nicolai (left) and Jenna Mumford (right), MSc research scholars from Victoria University of Wellington working on bloom-froming freshwater cyanobacteria.*

## Acknowledgements

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# 25 years of service enhancing the capacity to monitor and manage HABs

*Fig. 1. Participants from the first course held at the IOC Centre in Copenhagen in 1995*

The IOC Science and Communication Centre on Harmful Algae opened in May 1995 at the University of Copenhagen, Denmark. It was a new concept for the IOC to collaborate so closely with a national research institution to deliver international training, capacity development, coordination and support research initiatives as well as to function as a decentralized programme office of the IOC itself.

For the first decade, the Centre had a strong focus on assisting the least developed countries to build capacity for monitoring and managing HABs as the bulk of the funding was provided to the IOC by Danida, the Danish foreign aid programme. After then, activities diversified to more broadly underpin the activities of the Intergovernmental Panel on Harmful Algal Blooms (IPHAB) including the GEOHAB/GlobalHAB programmes, while training activities became targeted to the HAB community in general including an exam at the end of the course with an '*IOC Certificate of Proficiency in Identification of Harmful Algae*'. The course activity was later expanded to include close cooperation with the Marine Institute (Ireland) implementing the IPI, the International Phytoplankton Intercalibration scheme.

*Fig. 2. Jacob Larsen, driver behind the training courses offered by the Copenhagen Centre since the beginning (1995). In the photo, in action during a course preparing samples for the microscope.*

*Fig. 3. Santiago Fraga, who became one of the permanent teachers at the courses offered by the Centre in Copenhagen and Vigo, here during a taxonomy class.*

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

Some +700 people have attended training courses on HABs either at the Centre in Copenhagen or at courses held around the world. These courses have been driven by the scientists at University of Copenhagen including Jacob Larsen, Øjvind Moestrup, and Nina Lundholm and assisted by many dedicated colleagues from all over the world, nobody mentioned, nobody forgotten. It should be fair to say that the course activity has functioned as a major networking mechanism between the less and the more experience colleagues in the HAB community over the 25 years. Many research partners and new friends have been found among trainees and trainers.

What about the future? Both Jacob Larsen and Øjvind Moestrup seem to run on very strong batteries and the course activity is not planned to change, although in the short term amendments for the impact of COVID-19 need to be made. The Centre is working on a new version of the on-line / OceanTeacher part of the '*IOC Identification Qualification in Harmful Marine Microalgae*' to facilitate the consideration of course activity while international travel is restricted in the immediate future and perhaps beyond. Using a longer term perspective the shortage of in depth, detailed knowledge of species and identification based on morphology continues to diminish and there may be a day when there is no 'one stop shop' to get training on HAB species identification.

*Continued on next page*

# International Phytoplankton Intercomparison (IPI) exercise in abundance and composition of marine microalgae

Dear participants of the annual IPI (International Phytoplankton Intercomparison) exercise in abundance and composition of marine microalgae:

This note is to confirm that due to the ongoing pandemic across the globe caused by the SARS-COVID-19 virus, the IPI organising committee after discussion with our Advisory Group and partners have decided to cancel the IPI exercise for 2020. The proficiency testing scheme will continue from 2021 onwards. It is unfortunate that due to the current situation and the uncertainty surrounding it, the organisation of the exercise which includes the generation and posting of biological materials to many different laboratories around the world, development of educational tools like the *Oceanteacher* online test and organisation of the IPI workshop, which requires the presence of participants in a teaching room, has become too challenging and difficult to coordinate as a result of the ever changing situations in different countries. We are aware that many laboratories use the results of this exercise as part of their annual quality assurance requirements for their test method accreditation by their national accreditation bodies and also at the laboratory level for the ongoing competency of their analysts. This decision is not taken lightly and it is obviously going to affect everyone but I hope that given the circumstances, allowances should be made by the competent authorities to permit laboratories to maintain their accredited methods credentials until 2021 when we will resume the exercise.

The IPI exercise is organised by Rafael Salas (Marine Institute) and Jacob Larsen (IOC Science and Communication Centre on Harmful Algae) in collaboration with Claudia Delgado (IODE office). If you are interested in participating in 2021, you can register through our website *www.iphyi.org* next year and find all the information about the exercise. You can also e-mail me directly if you want to be included in future mailing about this test.

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The Centre is taking part in delivering on IOC's obligation to prepare the UN Decade of Ocean Science for Sustainable Development (2021-2030). The Decade may bring new opportunities for HAB science to be relevant to society, new demands for HAB expertise in order to reach the ambitious objectives of the Decade, and the Centre will strive to adapt to facilitate this.

We hope to work with all you for many years to come!

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*Henrik Enevoldsen, Head of the IOC Science and Communication Centre since its establishment*

# Canadian review: Marine harmful algal blooms and phycotoxins of concern to Canada

As has been reinforced all too well in recent months with the COVID-19 pandemic, the world is indeed interconnected. The international harmful algal bloom community recognized this early on, with a series of international conferences on HABs, starting in 1975 [1]. The International Society for the Study of Harmful Algae (ISSHA) was initiated in 1997. ISSHA made a commitment at the Florida international conference in 2002 to help develop a worldwide map of HABs and, beginning with the XII HAB conference (2006) in Copenhagen, ISSHA (along with the local organizing committee), also committed to help in the production of the proceedings of future international HAB meetings [2]. Also in 2002, the international HAB community developed the program Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) now called GlobalHAB [3,4].

Each country impacted by HABs has also developed national HAB programs. In Canada, the Phycotoxins Working Group (PWG) was established in 1988, by Fisheries and Oceans Canada (DFO), as a result of human poisonings by a novel phycotoxin (domoic acid) in late 1987, in eastern Prince Edward Island. One goal of the PWG was to "apprise senior management within DFO on harmful algae, including planning, coordinating and prioritizing research activities" [5]. This resulted in an unpublished review, written by the PWG in 1994, with the goal of informing management at DFO headquarters in Ottawa of the status and ongoing risks of marine HABs and phycotoxins in Canada.

Over the years since then, information was added to that review regarding new HAB events and novel phycotoxins discovered in Canada as discussed in a Canadian HAB Working Group meeting (Fig.1). The authorship was expanded to include 16 other scientists from DFO, the National Research Council of Canada, the Canadian Food Inspection Agency, the Canadian Museum of Nature, and Microthalassia Consultants Inc. (see Acknowledgements). This allowed the co-authors to contribute their individual expertise in the diverse fields of phytoplankton taxonomy, physiology, ecology, and the analytical chemistry of phycotoxins. The result is a comprehensive review of marine HABs and phycotoxins of concern to Canada [6]. This 322 page review contains sections on the history and distribution of HABs in Canada, the toxic and otherwise harmful phytoplankton that compose the HABs, and the phycotoxins that they produce. Links to Canadian reports in the ICES-IOC Harmful Algal Event Database (HAEDAT) since 1988 [7] are included. It also reviews HAB issues in Canada relating to climate change, ocean acidification, ships' ballast water, the Canadian Arctic, as well as monitoring programs and how they have changed over the years. Finally, it points out knowledge gaps and provides recommendations for further research. The text is supplemented by 13 tables, 13 figures, nine chemical structures of phycotoxins, and four appendices. A 126-page reference list contains links to PDF files or abstracts at journal websites. Below is an extended abstract of the Canadian HAB review:

*Fig. 1. Participants at the Third Harmful Algal Bloom Working Group Meeting (25-27 September 2018, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada), which included a discussion of the Canadian HAB review*

In Canada, as in many parts of the world, reports of marine harmful algal blooms (HABs) have increased over the past few decades. HABs are caused by the growth of certain phytoplankton that produce phycotoxins or otherwise cause harm. Phycotoxins are problematic not only to human health, but their cumulative effects are also stressors to marine ecosystems; some cause the mortality of marine fish, birds and mammals, including species designated at risk. Paralytic Shellfish Poisoning (PSP), caused by saxitoxin group toxins produced by *Alexandrium* spp. has been problematic for years along virtually the entire coast of British Columbia (BC), and on the east coast in the Bay of Fundy, the lower St. Lawrence Estuary, and the Gulf of St. Lawrence (Figs. 2-3). Since 1982, saxitoxin group toxins have been present sporadically in Newfoundland and Labrador (NL) and the Atlantic coast of Nova Scotia (NS). Amnesic Shellfish Poisoning (ASP, caused by domoic acid produced by *Pseudo-nitzschia* spp.) was identified for the first time worldwide following consumption of blue mussels (*Mytilus edulis*) from Prince Edward Island (PE) in 1987. Domoic acid has since been found in molluscan shellfish in the Bay of Fundy, New Brunswick (NB), NS, Quebec (QC), NL, offshore banks (Georges and Browns Banks), and in BC (Fig. 2). Diarrhetic Shellfish Poisoning (DSP, caused by okadaic acid group toxins produced by *Dinophysis* spp. feeding on ciliates and by benthic and epiphytic *Prorocentrum* spp.) was first recognized as a hazard in Canadian waters in 1990,

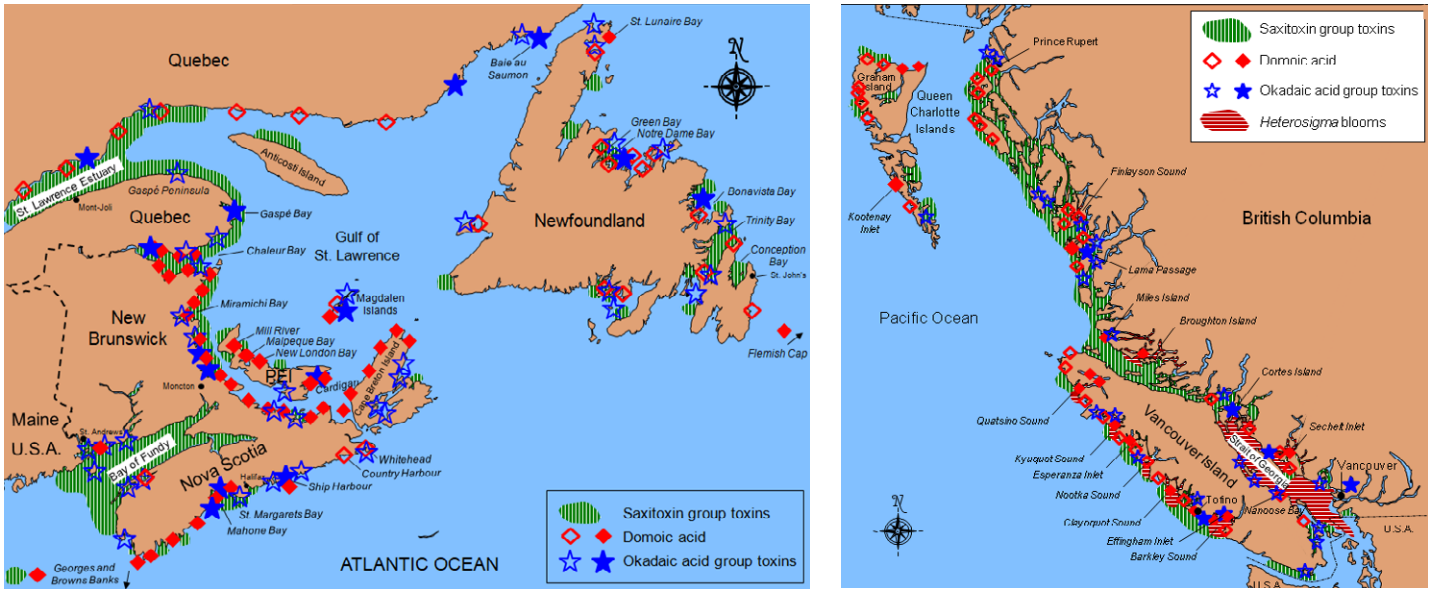


*Fig. 2. Maps showing the location of selected phycotoxins on the Canadian east (left) and west (right) coast. Symbols represent domoic acid and okadaic acid group toxins above (closed symbols) and below (open symbols) the regulatory action level. The green shaded areas show the distribution of saxitoxin group toxins. The red shaded areas in the map on the right show the distribution of fish-killing Heterosigma blooms.*

in NS. These toxins have since been found in the Bay of Fundy, the Gulf of St. Lawrence, QC, NL, and BC (Fig. 2).

Low levels of other phycotoxins that may cause harm to human health have recently been discovered in Canadian waters. These include pectenotoxins, yessotoxins and azaspiracids, as well as the cyclic imine group toxins (spirolide toxins, pinnatoxins, and gymnodimines). Pectenotoxins (produced by *Dinophysis acuminata*), spirolides (produced by *Alexandrium ostenfeldii*), and pinnatoxins (produced by *Vulcanodinium rugosum* in other parts of the world, but the Canadian producer has not yet been identified) have been detected in waters of both the Atlantic and Pacific coasts. Yessotoxins (produced by *Gonyaulax spinifera*, although not proven for Canadian waters, and *Protoceratium reticulatum*) are reported from NL, PE, NB, NS, and BC. Azaspiracids (produced by *Azadinium spinosum* and *A. poporum* in other parts of the world, but the Canadian producer has not yet been identified) have so far been found only in NL, NS, and QC. Gymnodimines (produced by *Karenia selliformis* and *A. ostenfeldii*, although both not proven for Canadian waters) are present in waters of NL, NS, QC, and BC. With the exception of pectenotoxins, no regulatory limits have yet been established for the above newer phycotoxins.

Multiple harmful algal species have been associated with fish-killing blooms in BC, including the diatoms *Chaetoceros convolutus* and *C. concavicornis*; the raphidophyte *Heterosigma akashiwo* (Fig. 4); the dinoflagellates *Margalefidinium polykrikoides* (formerly *Cochlodinium polykrikoides*), *M. fulvescens* and *Alexandrium catenella*; and the silicoflagellates (dictyochophytes) *Dictyocha fibula*, *Octactis speculum*, and *Pseudochattonella verruculosa*. Harmful species that have been associated with fish kills on the Atlantic coast include the diatoms *Eucampia zodiacus* and *Leptocylindrus minimus*, the dinoflagellate *A. catenella*, and the ciliate *Mesodinium rubrum* (Fig. 5). Several other algae *Chrysochromulina polylepis* (syn. *Prymnesium parvum*), *Gyrodinium aureolum* (now called *Karenia mikimotoi* in many regions), known to be problematic elsewhere in the world, are reported at low concentrations on both coasts.

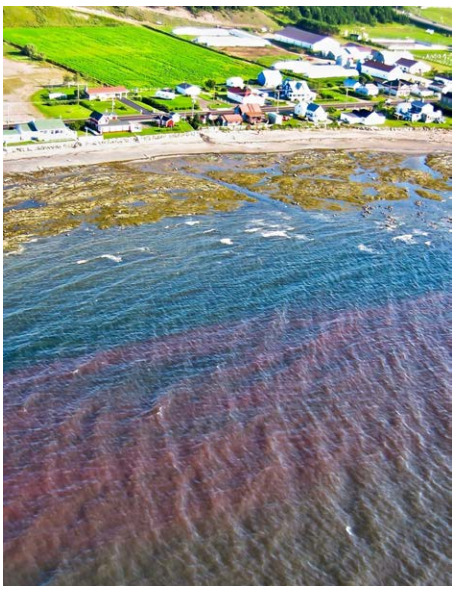

*Fig 3. Red tide of Alexandrium catenella (PSP toxin producer) previously called A. tamarense in the St. Lawrence Estuary near Ste-Flavie, Quebec, 2008. Photo courtesy of Michel Starr, Maurice Lamontagne Institute – DFO)*

The range of exotic toxic/harmful algae is expanding in Canadian waters due to introductions by ships' ballast water and spreading related to climate change and other natural and anthropogenic vectors. Canada's experience in dealing with toxic events has resulted in research and monitoring programs designed to understand HABs and to assist the fishing and aquaculture industries. In spite of decreases in research and phytoplankton monitoring efforts, consumers of molluscan shellfish are still protected by phycotoxin monitoring, which is conducted by the Canadian Food Inspection Agency. Nevertheless, novel phycotoxins and toxic algae will continue to be discovered. Questions remain about the mechanisms associated with the initiation and decline of some HABs, the source organisms of several of the phycotoxins, the role of toxins and other mechanisms of fish-killing HABs, and about the causes of inter-annual variability in HABs. Further study of novel toxigenic species and phycotoxins will be required to understand the degree of harm being done. HAB-related problems will be exacerbated with continued ship ballast water exchange and climate change.

*Fig. 4. Heterosigma akashiwo bloom in Kyuquot, British Columbia, 1996 (Photo courtesy of Nicky Haigh, Microthalassia Consultants Inc., Nanaimo, BC)*


*Fig 5. Red tide of the ciliate Mesodinium rubrum, potential prey of Dinophysis, in Passamaquoddy Bay, southwest NB, 1999 (Photo courtesy of Jennifer L, Martin, Saint Andrews Biological Station – DFO)*

The detection of domoic acid and the discovery of several toxigenic diatoms and dinoflagellates in the Canadian Arctic is of increasing concern because of the limited knowledge of HABs in this region. Continued vigilance and the maintenance of an effective capacity to manage developing problems via strategic research programs is essential. The goal of this document is to review Canadian marine harmful algal events and phycotoxins up to late 2018 (including relevant literature from Canada and internationally up to 2020) and to provide a foundation for any future research in this area.


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# ISSHA's Corner

**CHA 2021 Hybrid Conference, NEW DATE!!**

Dear ISSHA members and colleagues:

Due to the COVID-19 pandemic and following recommendations of the World Health Organization and National Health Authorities, the 19th International Conference on Harmful Algae has been postponed to October 10-15th, 2021.

We are currently working on changes for having a live and virtual hybrid meeting. The virtual meeting will be held in the same venue, the International Conference Center in La Paz, Baja California del Sur, Mexico. Our goal is to provide a high-quality conference to motivate future harmful algae research. We will keep you informed of any conference updates through our web page (www.icha2021.com ), email and social media.

All ISSHA members who renewed their membership after October 1st, 2019 will maintain their membership until the date of the conference. Please take the time to renew your ISSHA membership to allow for future student travel, awards and exciting speakers.

We appreciate your understanding and look forward to your participation.

*Christine Band-Schmidt, Chair of the Local Organizing Committee on behalf of the ISSHA Conference Organizing Committee*


**18th ICHA Proceedings. Harmful Algae 2018**

Dear ISSHA members and colleagues,

I am pleased to announce that the Proceedings of the International Conference on Harmful Algae, held in 2018 in Nantes, France, are now available on *the ISSHA website*.

*Ph. Hess [Ed]. Harmful Algae 2018 – from ecosystems to socioecosystems. Proceedings of the 18th International Conference on Harmful Algae. International Society for the Study of Harmful Algae, 2020. 214 pages.*

Thanks to Dr. Philipp Hess, IFREMER, and all his colleagues for a successful conference. Thanks to all the authors, reviewers and editors for their dedication to HAB science.

*Vera Trainer, ISSHA President*

United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

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The system will send back a mail to you with subject similar to 'auth 22d6dcd9 subscribe han' and with instructions to follow.

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