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

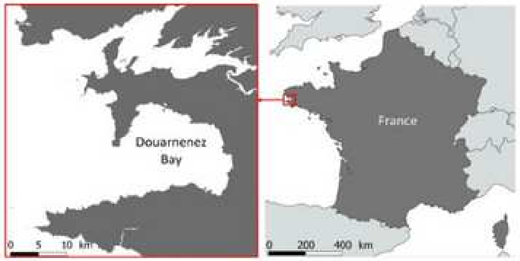

Fig. 1. Location of Douarnenez Bay (48° 5' 29" North; 4° 19' 51" West), Western French Atlantic coast.

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

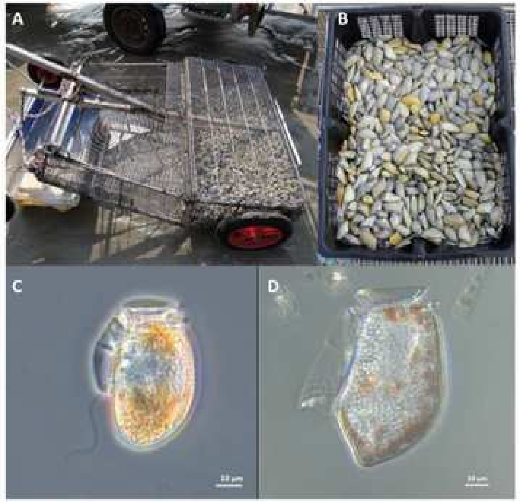

Fig. 2. (A) Sampling device to collect (B) Donax clams. Light micrographs of (C) Dinophysis acuminata and (D) Dinophysis acuta. (Photos A, B by Dominique Le Gal; C, D by Audrey Duval).

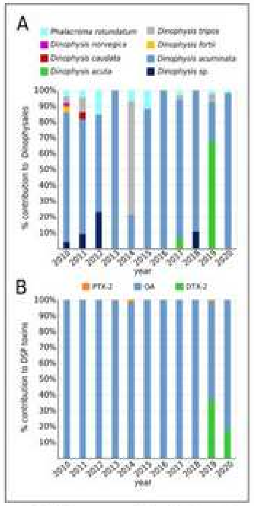

Fig. 3. (A) Percentage of Dinophysis species in Douarnenez Bay water samples between 2010 and August 2020. (B) Mean percentage of DSP toxins in Douarnenez Bay in Donax spp. between 2010 and August 2020.

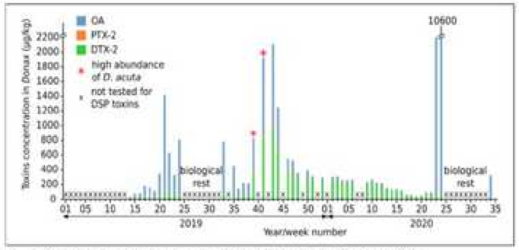

Fig. 4. Weekly lipophilic toxin concentrations in Donax spp. in 2019 and 2020.

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.

References

1. *Belin C & D Soudant D 2018. Trente années d'observation des microalgues et des toxines d'algues sur le littoral. Editions QUAE. https://archimer.ifremer.fr/doc/00478/58981/*
2. *Communauté de communes du pays de Châteaulin et du Porzay (CCPCP), Communauté de communes du pays de Douarnenez (CCDZ) (2011). Diagnostic hydrologique - Plan gouvernemental de lutte contre les algues vertes. http://www.sagebaiededouarnenez.org/site/ressources-et-documents/le-plan-gouvernemental-de-lutte-contre-les-algues-vertes/*
3. *REPHY 2019. REPHY dataset - French Observation and Monitoring program for Phytoplankton and Hydrology in coastal waters. 1987-2018 Metropolitan data . SEANOE. https://doi.org/10.17882/47248*
4. *REPHYTOX - 2019. REPHYTOX dataset. French Monitoring program for Phycotoxins in marine organisms. Data since 1987 . SEANOE . https://doi.org/10.17882/47251*

Authors

Amélie Derrien, Aouregan Terre-Terrillon, Audrey Duval, Anne Doner & Nicolas Chomérat, Ifremer - LER Bretagne Occidentale, Place de la croix, 29900 Concarneau, France

Email corresponding author:
amelie.derrien@ifremer.fr

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].

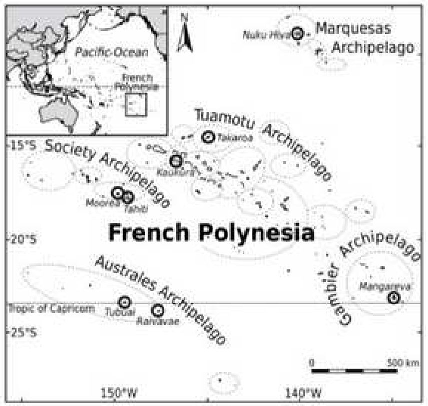

Fig. 1. Map of French Polynesia showing the five archipelagoes. Islands where samples have been collected for this study are circled and their names are in italics.

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]. Morphologically, 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.

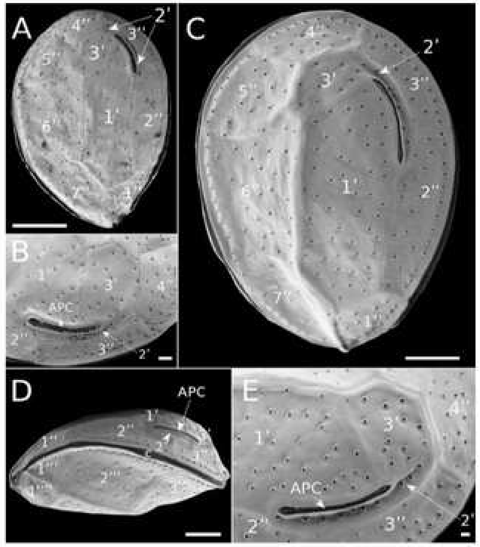

Fig. 2. Scanning electron micrographs of two previously unreported Ostreopsis species in French Polynesia. (A) Apical view of O. rhodesiae. (B) Detail of the apical pore complex (APC) of O. rhodesiae. (C) Apical view of Ostreopsis sp. 6. (D) Left lateral view of Ostreopsis sp. 6 showing the undulated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

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

These data were obtained in the framework of the research program TATOO funded by the "Délégation à la Recherche de Polynésie française" (Conv. n° 02400/MTF/REC of April 9th, 2018). We are grateful to Kevin Henry, André Ung, Mayalen Zubia and Christophe Vieira for help in field samplings.

References

1. *Chinain M et al 2020. Harmful Algae, in press*
2. *Yasumoto T et al 1977. Bull Japan Soc Fish 43: 1021-1026*
3. *Adachi R and Fukuyo Y 1979. Bull Japan Soc Sci Fish 45: 67-71.*
4. *Fukuyo Y 1981. Bull Jap Soc Sci Fish 47: 967-978*
5. *Bagnis R et al 1985. In: Anderson DM et al (eds), Toxic Dinoflagellates, Elsevier, New York, pp 177-182*
6. *Tester PA et al 2020. Harmful Algae 91; 101655; doi:10.1016/j.hal.2019.101655*
7. *Chomérat N et al 2019. Harmful Algae 84: 95-111*
8. *Verma A et al 2016. Harmful Algae 60: 116-130*
9. *Chomérat N et al 2020 Harmful Algae 98; 101888; doi:10.1016/j.hal.2020.101888*
10. *Schmidt J 1901. Bot. Tidsskr. 24: 212-221*

Authors

Nicolas Chomérat & Gwenael Bilien, IFREMER, LER Bretagne Occidentale, Place de la Croix, F-29900 Concarneau, France

Damien Réveillon & Fabienne Hervé, IFREMER, Phycotoxins Laboratory, PO Box 21105, F-44311 Nantes, France

Jérôme Viallon, H. Taiana Darius & Mireille Chinain, Institut Louis Malardé, Laboratory of Marine Biotoxins, UMR 241-EIO, PO Box 30, 98713 Papeete, French Polynesia

Email corresponding author:
nicolas.chomerat@ifremer.fr