

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

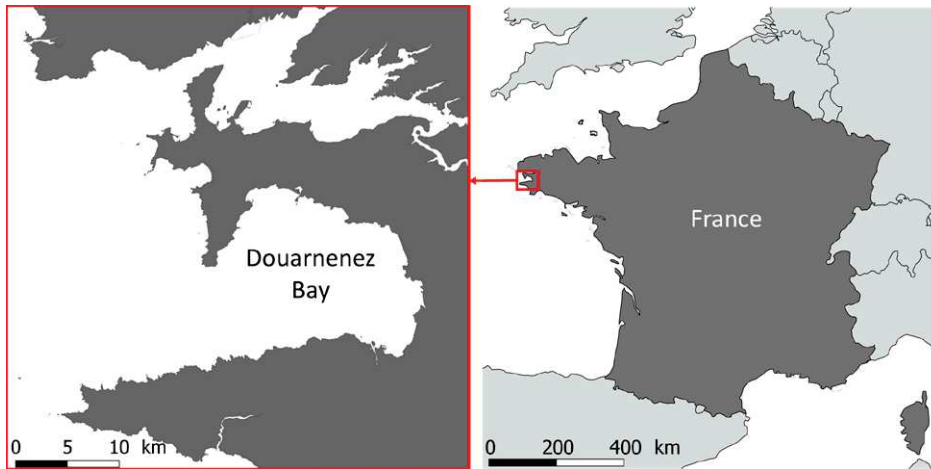


Fig. 1. Location of Douarnenez Bay (48° 5' 29" North; 4° 19' 51" West), Western French Atlantic coast.

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 spectrometry 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 km² (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 10² to 12 x 10³ cells L⁻¹. The predominant toxin associated with this taxon was okadaic acid (OA) (Fig. 3 B). The maximum OA concentration

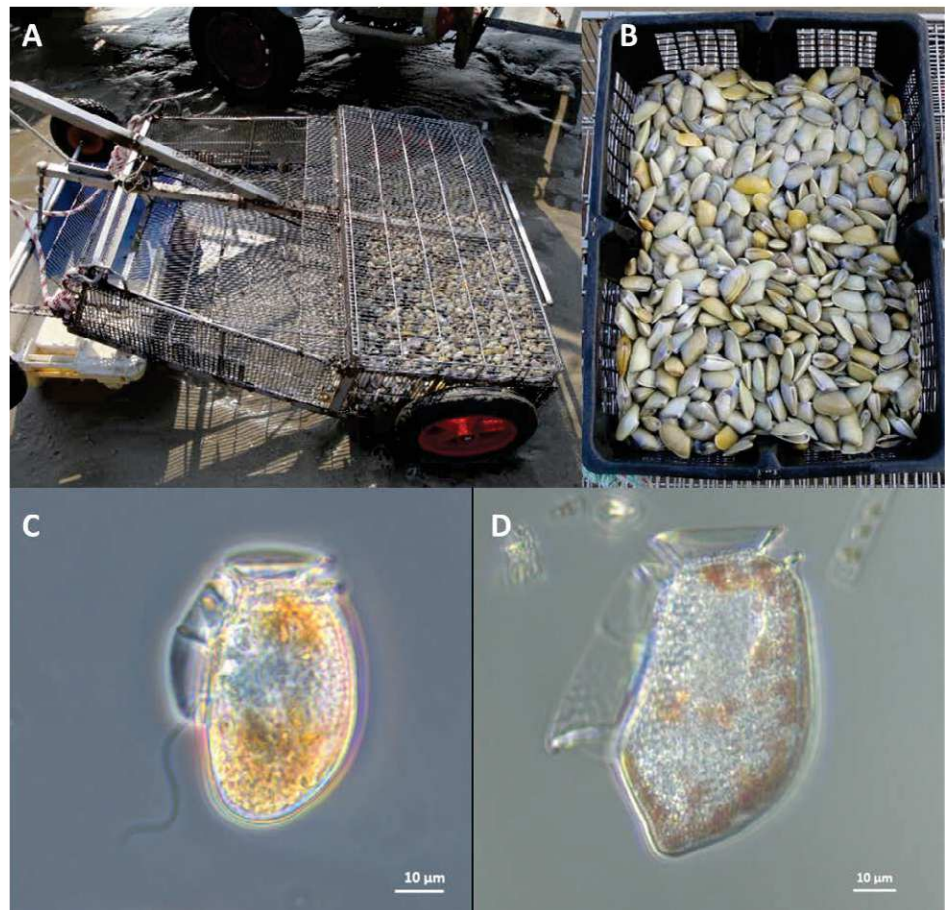


Fig. 2. (A) Sampling device to collect (B) *Donax* clams. Light micrographs of (C) *Dinophysis acuminata* and (D) *Dinophysis acuta*. (Photos A, B by Dominique Le Gal; C, D by Audrey Duval).

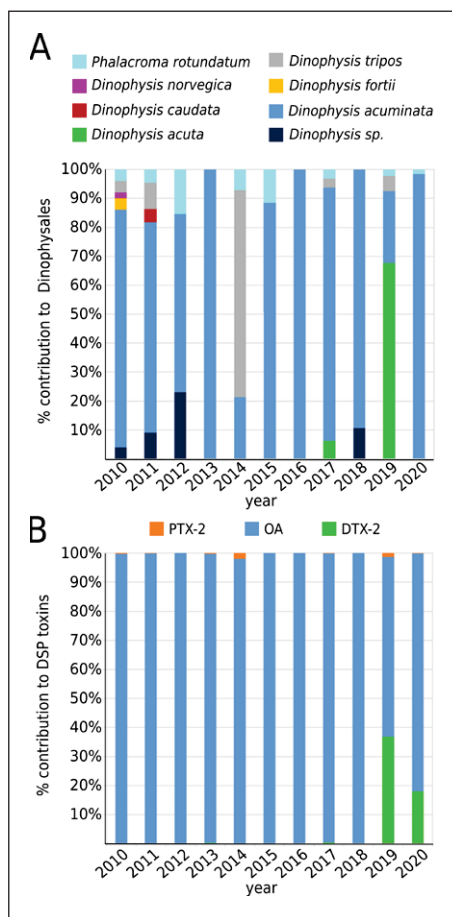


Fig. 3. (A) Percentage of *Dinophysis* species in Douarnenez Bay water samples between 2010 and August 2020. (B) Mean percentage of DSP toxins in Douarnenez Bay in *Donax* spp. between 2010 and August 2020.

recorded in *Donax* spp. was 9,853 $\mu\text{g kg}^{-1}$ 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 5.5×10^3 cells L^{-1} (week 39) and 3.3×10^3 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.*

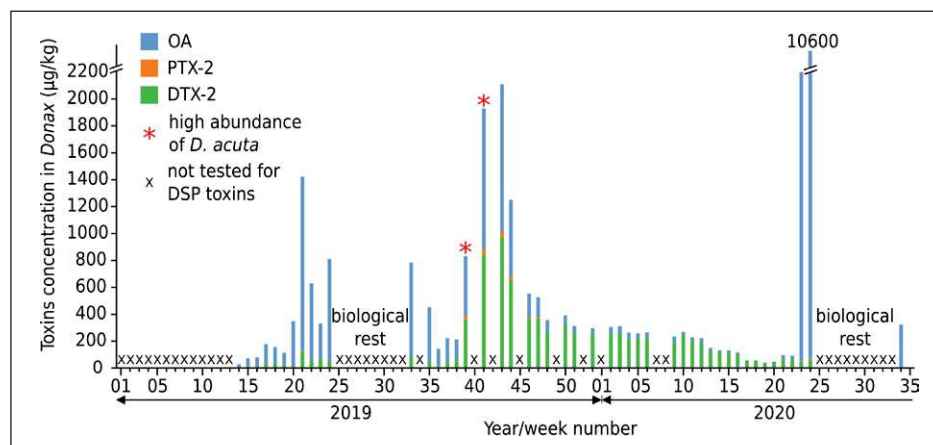


Fig. 4. Weekly lipophilic toxin concentrations in *Donax* spp. in 2019 and 2020.

acuta was first detected (5.5×10^3 cells L^{-1}), DTX2 and OA concentrations in *Donax* spp. were $356 \mu\text{g kg}^{-1}$ and $443 \mu\text{g kg}^{-1}$, respectively and in week 41, after the second detection of *D. acuta* (3.3×10^3 cells L^{-1}), $840 \mu\text{g kg}^{-1}$ and $1,045 \mu\text{g kg}^{-1}$ respectively. Finally, in week 43, maximum concentrations of DTX2 ($974 \mu\text{g kg}^{-1}$) and OA ($1089 \mu\text{g kg}^{-1}$) 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 $160 \mu\text{g kg}^{-1}$ (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

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