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Three decades of data on phytoplankton and phycotoxins on the French coast: Lessons from REPHY and REPHYTOX

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Abstract :

In France, REPHY (Observation and Surveillance Network for Phytoplankton and Hydrology in coastal waters) and REPHYTOX (Monitoring Network for Phycotoxins in marine organisms) have been contributing to long-term time series on ocean health for more than 30 years. The aim of this paper is to describe these networks and to highlight their key results. Over the last 20 years, phytoplankton flora analysis on French coasts from the Channel to Mediterranean has shown that the five “emblematic” taxa are Chaetoceros, Skeletonema, Cryptophyceae, Leptocylindrus and Pseudo-nitzschia. The latter, together with the taxa of interest Dinophysis + Phalacroma, Alexandrium, and Karenia, have been consistently recorded along the entire French coastline. However, when taking into account frequency of occurrence some taxa exhibit more distinct geographical distributions. In particular, the occurrence of Phaeocystis appeared to be strongly specific to the northern coasts of the Channel. French coasts have been regularly affected since the 1980s by the presence of toxins in bivalve molluscs, leading to bans on fishing and sale of shellfish during periods of varying duration. Three categories of toxins were involved. PST and AST were absent from the French coasts, respectively before 1988 and 2000. DST (Diarrheic Shellfish Toxins) have affected many areas along the whole coast every year since 1987. For PST (Paralytic Shellfish Toxins), only a few areas have been affected, sometimes sporadically, since 1988 in the Channel, 1993 in the Atlantic, and 1998 in the Mediterranean. Many areas have been impacted since 2000 by AST (Amnesic Shellfish Toxins) episodes, mainly affecting scallops in the Channel and on Atlantic coasts. The patterns of change of shellfish toxicity episodes showed no real trend in any province over the entire period 1987–2018.

Highlights

► REPHY and REPHYTOX have been contributing to ocean survey for more than 30 years. ► Five taxa are "emblematic" of phytoplankton flora from the Channel to Mediterranean. ► Four harmful taxa have been consistently recorded along the entire French coastline. ► Episodes of toxicity in shellfish mainly started in the 1980s and 90s. ► Patterns of change of toxicity episodes have not shown a real trend for 30 years.

Keywords : Monitoring network, Phytoplankton time-series, Dominant species, DST-Diarrheic, Shellfish Toxins, PST-Paralytic Shellfish Toxins, AST-Amnesic Shellfish Toxins

Terminology

Taxonomic Units (TU): taxa clusters, most often groups of genera and / or groups of species, described to allow data processing.

Dominant taxa: the taxa most present on the French coast, calculated by a method derived from the Sanders index (1960).

Emblematic taxa: some of the most dominant taxa + some toxic or harmful taxa that are not dominant. These taxa are considered emblematic either on the whole French coast or in one of the three provinces (Channel, Atlantic, Mediterranean).

1 Introduction

Phytoplankton provide key ecosystem services in terms of the functioning of the biological pump, regulation of climate, generating half of the oxygen in our atmosphere, and as the base of marine food webs. Numerous global changes since the beginning of the industrial era, such as eutrophication, warming, acidification and deoxygenation of the ocean, represent key stressors for marine ecosystems. Phytoplankton responses to these include: (i) increase in phytoplankton biomass and oxygen depletion in bottom waters (Diaz and Rosenberg, 2008); (ii) changes in structure of phytoplankton communities in response to changes in nitrogen / phosphorus / silica ratios (Philippart *et al.*, 2000); (iii) increased frequency of harmful algal blooms (Hallegraeff, 1993; Anderson *et al.,* 2002; Anderson, 2009; Wells *et al.* 2015); (iv) alteration of the phenology of phytoplankton communities (Leterme *et al.*, 2008). Human health can be impacted by species that produce toxins that are dangerous for humans who consume seafood, as well as environmental impacts from species that are toxic or harmful for the marine fauna and flora. For all these reasons, knowledge of phytoplankton dynamics is essential. Most natural ocean processes vary on annual to decadal timescales and to identify shifts resulting from climate change, observations need to span at least 30 or preferably 50 years or more (Koslow and Couture, 2013). In this context, the acquisition of long-term series of phytoplankton species has become a priority in the monitoring of the marine environment, knowing that long-term series on phytoplankton are not common worldwide. The study of these issues is already benefiting from the availability of long-term time series from coastal sites subject to contrasting environmental and human pressures at a global scale. For example: (i) plankton observation data produced since 1931 by the Continuous Plankton Recorder (CPR), initially deployed in the North Sea and then extended to the North-East Atlantic and now globally, (ii) the data series since 1962 on plankton 'Helgoland Roads', located in a German archipelago in the southeast of the North Sea.

In France, the creation in 1984 of the monitoring network REPHY-REPHYTOX by Ifremer was part of this process. The objective of REPHY is to acquire data of the biomass, abundance and composition of marine phytoplankton in coastal and lagoon waters, as well as the associated hydrological parameters. These data cover in particular the spatio-temporal distribution of phytoplankton species, and the identification of exceptional blooms. The objective of REPHYTOX is the detection and monitoring of the three categories of toxins that can accumulate in bivalve molluscs, which are regulated at European level: lipophilic toxins (including DST), PST and AST, which are respectively responsible for the following human syndromes: DSP (Diarrheic Shellfish Poisoning), PSP (Paralytic Shellfish Poisoning) and ASP (Amnesic Shellfish Poisoning). The two networks are closely associated, since the monitoring of toxic phytoplankton by REPHY is used for triggering toxin analysis by REPHYTOX and for a better understanding of shellfish contamination. REPHY is divided into three components: Observation, Surveillance and Health (Belin and Soudant, 2018). The aim of the Observation component is to seek answers to research questions such as: (i) what are the responses of phytoplankton communities to environmental changes (e.g. Hernández-Fariñas et al., 2014); (ii) participate in the definition of phytoplankton ecological niches (Hernández-Fariñas *et al.,* 2015); (iii) detect variations in phenology (Guallar *et al.,* 2017); (iv) participate in the characterization of traits and functional groups (David *et al.,* 2012). The main aim of the Surveillance component is to participate, in addition to the Observation component, in the assessment of the quality of the coastal waters through indicators, in particular those described that meet the requirements of the European directives WFD (Water Framework Directive) and MSFD (Marine Strategy Framework Directive). The purpose of the Health component is to participate in the triggering of shellfish sampling carried out within the framework of REPHYTOX network, by supplementing the results already acquired on the toxic species by the two other components. REPHYTOX monitoring applies to shellfish harvested for professional purposes in their natural environment i.e. in production areas and natural populations, and in professional fishing zones. In addition to providing the necessary information to the authorities responsible for managing the public health risk, this monitoring system makes it possible to acquire scientifically interesting data.

The purpose of this article is to present a comprehensive review of the data acquired since 1987: (i) on the phytoplankton of the French coast, with respect to the most dominant taxa and a survey of the geographical distribution of some toxic or harmful species; (ii) on the three categories of phycotoxins most widespread in France, with the temporal patterns of change of episodes of toxicity, and their geographical distribution. Data presented here do not include those from the French overseas departments/areas.

2 Material and methods

The REPHY and REPHYTOX monitoring networks were set up by Ifremer in 1984, with the first data available from 1987. All operations relating to REPHY and REPHYTOX, from sampling to analysis, were carried out using a quality approach, the main components of which were: a general quality assurance process, the accreditation or official approval of laboratories, documents and methods standardized at national level, regular performance testing of measuring equipment, intercalibration and inter-laboratory comparability tests. Methodological support, expertise and organization of training for staff were provided by experts qualified in the relevant fields.

2.1 Sampling strategies

REPHY and REPHYTOX spread over the whole French coastline. The three marine provinces of France are: the Atlantic Ocean, the Channel and the Mediterranean (see Fig. 1). The Channel is an epicontinental sea of the Atlantic Ocean, located in North-West Europe and separating France from the United Kingdom; the Eastern Channel is one of the busiest maritime areas of the globe; the water of this zone is, because of the currents among most important in the world, very turbid, while remaining oxygenated. The Mediterranean Sea is an almost entirely closed intercontinental sea, bordered by the coasts of Southern Europe, North Africa and West Asia; its opening to the Atlantic Ocean by the Strait of Gibraltar is 14 kilometers wide.

For REPHY, the Observation component comprised 36 sampling stations (Fig. 1). The Surveillance component complemented this system with another 116 stations distributed in water bodies not covered by the Observation component. The Health component included 71 additional stations with strictly health objectives, but also used data from 41 stations related to the Observation or Surveillance components. The sampling strategy consisted of *in situ* measurements, and water samples for microscopic analysis of phytoplankton and for selected additional analyses. Sampling and measurements were carried out under specific conditions, particularly at 1 m below the surface (dissolved oxygen was also measured at the bottom) and within 2 hrs either side of high tide in the Channel and the Atlantic. The sampling periods and frequencies, as well as the parameters to be measured, were different depending on the REPHY component, and the type of water body. For REPHY Observation, sampling was done all year round, once a fortnight. The mandatory parameters were: *in situ* measurements (temperature, salinity, turbidity, dissolved oxygen), microscopic analysis of all phytoplankton ('total flora'), chlorophyll *a*, pheopigments and nutrients. Dissolved oxygen was only measured in Summer from June to September. Phytoplankton pigments were also analyzed on samples from a selection of stations spread along the whole coastline. For REPHY Surveillance, sampling was generally done all year round, once a month. For most stations, the mandatory parameters were: *in situ* measurements, microscopic analysis of a selection of phytoplankton species ('targeted flora'), chlorophyll *a*, pheopigments and nutrients. However, some analyses were limited to one period of the year: (i) for Channel and Atlantic coasts, chlorophyll *a* and nutrients were measured respectively from March to October, and from November to February, (ii) dissolved oxygen was measured from June to September. Otherwise, for a few stations in Channel or Atlantic transitional water bodies, only *in situ* measurements and nutrient analysis were performed. In the Mediterranean lagoons, an estimation of nano- and pico-phytoplankton by flow cytometry was made in addition, from June to August. For REPHY Health, the sampling period at strictly health-related stations was dependent on the context: all year round, or over a limited period. The frequency was once a week. The only mandatory parameter was the microscopic identification of toxic phytoplankton species ('toxic flora'). At the Observation and Surveillance components stations identified as being useful for the Health component, sampling could possibly be carried out for additional toxic flora during the weeks without scheduled sampling.

For REPHYTOX, nearly 300 shellfish sampling stations could potentially be used, with mussels at almost half of the stations, as well as oysters, scallops, clams, cockles or various other shellfish. REPHYTOX sampling stations were in some cases in common with those of REPHY. Alert thresholds, based on historical data, were defined for the phytoplankton toxic species currently occurring on French coasts. Their detection by the REPHY network above these thresholds triggered toxin monitoring in shellfish on a weekly basis. These alert thresholds were: (i) for diarrheic shellfish toxin producing species: as soon as *Dinophysis* was present, whatever the species, 10,000 cells per liter for *Gonyaulax spinifera*, *Lingulodinium polyedrum*, *Protoceratium reticulatum* or *Prorocentrum lima* (when present in the water column); (ii) for paralytic shellfish toxin producing species: 10,000 cells per liter in areas usually affected by *Alexandrium minutum*, between 1,000 and 5,000 cells per liter in areas usually affected by *Alexandrium pacificum* according to the zone, 10,000 cells per liter for unidentified *Alexandrium* species; (iii) for amnesic shellfish toxin producing species: 300,000 cells per liter for the less toxic *Pseudo-nitzschia* species, 100,000 cells per liter for the most toxic; the difference between these two groups of species was made from their general form: broad or slender for the most toxic (including e.g. *P. australis*, *P. fraudulenta*, *P. multiseries*, *P. pungens*), thin or sigmoid for the least toxic (including e.g. *P. delicatissima*, *P. multistriata*); the notion of 'more or less toxic' is deduced from the history observations made in France, and is subject to evolution. However, there were two cases where the monitoring of toxic phytoplankton was not reliable enough to ensure the absence of toxins in shellfish. Firstly, lipophilic toxins were sometimes observed in shellfish before the presence of *Dinophysis* was detected; the search for these toxins was therefore systematically carried out in the 'risk zones' and during 'risk periods' defined using the history of results over the last three years. Secondly, offshore and deep-sea shellfish deposits were systematically monitored for all three toxin categories every fortnight, one month before and during the entire shellfish harvesting period. Mussels (*Mytilus edulis, M. galloprovincialis*) were considered as sentinels for lipophilic toxins, since historical data showed that they were always contaminated earlier than all other shellfish. If they were present in a production area, the mussels were analysed first, and the other shellfish as soon as the mussels became toxic. This was not the case for PST and AST episodes: all shellfish species were then monitored simultaneously.

2.2 Measured parameters and methods

For REPHY, the identification and count of phytoplankton cells under an optical microscope was performed at most REPHY sampling stations, with the exception of: (i) those located in turbid waters or too far upstream in a river, (ii) some stations located in Mediterranean lagoons for which phytoplankton was measured by flow cytometry. Phytoplankton data was grouped into three different categories: (i) 'total flora' corresponded to the identification and count of all phytoplankton taxa identified using light microscope; (ii) 'targeted flora' corresponded to the identification and count of a targeted list of species, including those in high concentrations and those which were known to be toxic or harmful; (iii) 'toxic flora' corresponded to the identification and count of the few species that produced toxins that could accumulate in shellfish. The method used was that of Utermöhl (1931). The identification of phytoplankton was based on the WoRMS (World Reference Marine Register) and had to be at the most precise taxonomic level (species or genus) if possible, and if not, at a higher taxonomic level (family, class, etc.). The analysis by flow cytometry, according to the Vaquer *et al.* (1996) method, provided functional groups for nano- and pico-phytoplankton. Chlorophyll *a* and pheopigments were essentially analyzed by monochromatic spectrophotometry or fluorimetry (Aminot and Kérouel, 2004). The measured nutrients were:ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), silicate ($SiOH_4$). The ammonium concentration was determined by flow fluorimetry, and the concentration of the other four nutrients by flow spectrophotometry (Aminot and Kérouel, 2007). In addition to phytoplankton observations, the measurement of physical and chemical parameters (i.e. temperature, salinity, turbidity and dissolved oxygen) were generally measured *in situ* using sensors, with a multi-parameter probe.

For REPHYTOX, detection and quantification of phycotoxins was carried out on the total flesh from live shellfish according to the official European methods. These also describe regulatory safety thresholds not to be exceeded for shellfish to be considered consumable. Lipophilic toxins including DST have been measured by Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS / MS) since early 2010 (Anonymous, 2015). They had been previously detected by mouse bioassay according to the method of Yasumoto *et al.*, (1984 modified). These results were therefore qualitative until 2009, and expressed in terms of 'favorable' or 'unfavorable'. The results were quantitative from 2010 and included quantification for each of the molecules of the groups of regulated toxins. The regulatory thresholds were (expressed per kg of shellfish flesh): (i) 160 µg of OA equivalent for the OA+DTX+PTX group (okadaic acid + dinophysistoxins + pectenotoxins); (ii) 160 µg of AZA equivalent for AZA (azaspiracids); (iii) 3750 µg of YTX equivalent for YTX (yessotoxins). Each of the constitutive toxins in these groups was corrected by a TEF (Toxic Equivalent Factor) to account for its potential toxicity. The quantification of PST was carried out by mouse bioassay, calibrated and validated to give a quantitative result (Anonymous, 2014). The regulatory threshold was 800 µg STX (saxitoxin) equivalent per kg of shellfish flesh. AST were measured by High Performance Liquid Chromatography with Ultra Violet detection

(HPLC/UV) (Anonymous, 2008). The regulatory threshold was 20,000 µg DA (domoic acid) equivalent per kg of shellfish flesh. For PST and AST, the results recorded in the REPHYTOX data did not include detailed results for each constitutive toxin.

2.3 *Data: storage, availability, communication*

All REPHY and REPHYTOX results have since 1987 been recorded in the national 'Quadrige' database, in compliance with a quality process, the main characteristics of which are: administration of the database by a dedicated data management team, mandatory training and agreement for the use of Quadrige, entering the results according to a user manual, compulsory checks and validation of the data entered, a quality process implemented *a posteriori* on the data in order to provide, for each result, information on the quality (good, doubtful or erroneous). Access to the raw data of Quadrige is restricted to authorized users, but the validated data are public, associated with a DOI (Digital Object Identifier), and accompanied by user manuals in English: REPHY (2017), REPHYTOX (2017), with the respective DOI: doi.org/10.17882/47248, doi.org/10.17882/47251. The datasets used for the presentation of the results below were all extracted from the Quadrige database.

2.4 Data presented and processing

Results presented at national scale were always aggregated geographically according to Quadrige's internal 'marine areas' reference system. This defines 123 contiguous marine areas: 38 for the Channel, 54 for the Atlantic and 31 for the Mediterranean. Phytoplankton taxa were always grouped in 'Taxonomic Units' (TU). The REPHY data were composed of more than 500 different taxa over the entire period 1987-2018, including more than 200 species or groups of species, more than 200 genera or groups of genera, and about 40 families. To partially address the difficulty of processing such heterogeneous data, Hernández-Fariñas *et al.* (2014) proposed using taxa clusters and described them as

Taxonomic Units, which were most often groups of genera and / or groups of species. The list was thus reduced to less than 250 TU.

Two characteristics of phytoplankton data for the last 20 years (1999-2018) are presented here: dominance and abundance. For the determination of 'dominant' TU, only 'total flora' data were taken into account for this calculation. The dominance measure used here was derived from the Sanders index (1960). This is determined by classifying the species from 1 to 10 by abundance in each sample. A rank of 1 receives a value of ten points, a rank of 2 nine points, etc. A rank of 10 receives one point. The points are summed on all samples: the higher the sum of points, the more dominant is the species. This approach consisted on the one hand of taking abundance into account by using relative numerical abundance of species in samples instead of points, and on the other hand considering all species and not only the ten most abundant ones. The summary of results for TU abundance was based on the 90th percentile, encompassing the spread of data omitting highly skewed values (Devlin *et al.*, 2014), computed with TU occurrence abundance during the considered period and marine area. These values were represented on a map as disks with a diameter proportional to their abundance class on a log 10 scale. The number of TU occurrences was taken into account through the transparency of the disk color varying linearly from transparent (i.e. 1 occurrence) to opaque (i.e. 240 occurrences, that is, identified each month during 20 years). Six TU were chosen according to their toxicity or harmfulness. Three of these TU included species that produced toxins that were dangerous to shellfish consumers (*Dinophysis + Phalacroma*, *Alexandrium* and *Pseudo-nitzschia*). The other three TU contained species that already led to mortalities of marine fauna in France: (i) *Karenia mikimotoi*, a producer of hemolytic ichthyotoxins; (ii) *Phaeocystis*, via mechanical action with production of foams; (iii) *Lepidodinium chlorophorum* which, by its capacity to develop rapidly in abundant quantities, has regularly led to anoxia in the marine environment .The results on toxins were summarized, on the one hand temporally, and on the other hand spatially. For the temporal dimension, a toxicity episode of a given toxin (i.e. DST, PST or AST) was defined by its occurrence in shellfish above the European regulatory threshold. For each given year and toxin, a marine area was considered affected by the given toxin if at least one toxicity episode was recorded. Then, to compare the number of affected areas by province, year and toxin, sums were divided by the number of marine areas of the corresponding province and plotted as stacked bar charts. For the spatial dimension, three maps were produced (one per toxin) showing for each marine area the number of years affected by at least one toxic episode in the year. In order to achieve more relevant comparison between the respective impacts of the three categories of toxins, the focus for these maps was on the period 2000-2018 (the PST and AST being less or not present before 2000). Data processing, tables and maps were made with R (2018) and QGIS (2018).

3 Results

Most of the location names cited in the text appear in Fig. 1.

3.1 Phytoplankton

Table 1 details the most dominant TU for the period 1999-2018. It shows that five TU can be considered as the most emblematic of the French coastline as a whole: *Chaetoceros*, *Skeletonema*, Cryptophyceae, *Leptocylindrus*, *Pseudo-nitzschia.* These always belong to the group of the ten most dominant TU, whatever the geographical region. Among the target TU, *Phaeocystis* was also an element of interest although it has never been detected on Mediterranean French coast. It reached the 64th dominance rank in the Atlantic, but was the second most dominant TU on the French coast of the Channel. *Lepidodinium*, which is another harmful TU of interest, has also never been observed on the Mediterranean French coasts. Unlike *Phaeocystis*, it is more dominant on the Atlantic coasts than in the Channel. The three other toxic or harmful TU of interest, i.e. *Dinophysis* + *Phalacroma*, *Alexandrium* and *Karenia,* are towards the bottom of the ranking, respectively, 47th, 49th and 61st. Over the same period of 20 years, Fig. 2 provides a summary of the geographical distribution of some of these TU, chosen because they include toxic or harmful species. The geographical distribution of *Pseudo-nitzschia* shows that it was regularly present along the entire coastline at high concentrations. *Dinophysis* + *Phalacroma*, *Alexandrium* and *Karenia* have been observed in all marine provinces. *Dinophysis* + *Phalacroma* occurred mostly in the Bay of

Seine, in western and southern Brittany, and in the lagoons of the western Mediterranean or Corsica. *Alexandrium* appeared more frequently in Brittany and in the lagoons of the western Mediterranean. *Karenia* was observed everywhere, but its regular presence was only observed in certain areas, especially in western and southern Brittany, in low concentration. *Phaeocystis* was observed mainly in the Channel and in southern Brittany, with the highest concentrations along the northern parts of the French coasts. *Lepidodinium* was very little present in the Channel and was absent in the Mediterranean. It was mainly present in southern Brittany and around the Loire estuary.

3.2 Toxins

Fig. 3 shows that on the Atlantic coast, the percentage of affected areas was almost always greater than in the other provinces. The appearance of AST episodes was observed on this coast since the mid-2000s. This pattern is also found in the Channel, on a smaller scale. DST episodes do not seem to have increased or decreased over these 32 years, regardless of the province. PST episodes occurred on all three provinces, but the percentage of affected areas has not increased significantly since their appearance respectively in 1988 in the Channel, and 1998 in the Mediterranean. Fig. 4 shows the geographical distribution and the recurrence of episodes of toxicity during the last 19 years, by toxin, and by area. It shows that many areas were regularly affected by DST episodes, especially in Normandy, in western and southern Brittany and in several regions of the Atlantic coast, on the Mediterranean coast, and in lagoons of the western Mediterranean and Corsica. Conversely, whole regions were spared from DST episodes, especially along the northern coast of France, and along the Channel. With regard to PST episodes, a few areas were affected over the period, sometimes sporadically. The two most frequently affected areas were: Brest harbor in western Brittany, and Thau lagoon in the Mediterranean. The AST episodes showed a pattern that was different from that of DST and PST, with a large number of affected areas but fewer than for DST. The most frequently affected areas over the period were: the Bay of Seine in Normandy, and the western and southern coasts of Brittany.

Table 2 summarizes the information on the relative weight of the shellfish affected by the episodes of toxicity. In some cases, the different species of a genus are grouped, either because some species hybridise with each other or because they are difficult to identify. This is the case of: (i) *Mytilus*, the species occurring in France being *M. edulis* (in Channel and Atlantic), *M. galloprovincialis* (rather Mediterranean), or a hybrid of the two; (ii) *Donax*, which includes *D. trunculus* and *D. vittatus*; (iii) *Ruditapes*, which includes *R. decussatus* and *R. philippinarum* or their hybrids; (iv) *Mimachlamys* which includes the species *M. varia*. Table 2 shows that: DST episodes regularly affected a wide variety of shellfish especially in the Atlantic, PST episodes affected only a few species of shellfish, while AST episodes mainly affected scallops, especially in the Channel. This inventory should be qualified by the fact that the small number of areas affected by PST reduced the range of shellfish species impacted.

4 Discussion

4.1 Phytoplankton

The following are examined and discussed: the most dominant TU (see Table 1), then the TU with toxic or harmful species, some of which are mapped in Fig. 2.

The TU *Chaetoceros*, *Skeletonema*, Cryptophyceae and *Leptocylindrus* exhibited approximately the same geographical pattern as that of *Pseudo-nitzschia* (and are therefore not mapped in Fig. 2): in terms of presence on all marine areas of the coast, at high or very high concentrations. *Chaetoceros* included species with barbs on their setae likely to cause lesions on fish gills and therefore mortalities: in France this was the case in 2010 in western Brittany (Lassus *et al.*, 2016). *Skeletonema* and *Leptocylindrus* are not known to be harmful in France. The REPHY data showed that in terms of annual maxima per marine area: (i) *Chaetoceros* regularly exceeded 10 million cells per liter, particularly in the Channel; (ii) *Skeletonema* often exceeded 1 million cells per liter, and often 10 million in the Mediterranean, and even reached 550 million in northwestern Brittany in 2000; (iii) Cryptophyceae quite often exceeded 1 million; (iv) *Leptocylindrus* often exceeded 1 million cells per liter, in all provinces. Blooms greater than 1 million for these four TU were generally observed from March to October in the Channel and Atlantic, and in all seasons in the Mediterranean. For the TU containing toxic or harmful species, and mapped in Fig. 2, they systematically include all species belonging to the TU, regardless of their harmful potential. However, the geographical distribution of these TU provides in most cases interesting information in terms of risk.

For *Pseudo-nitzschia, which* was regularly present everywhere (Fig. 2), the pattern was similar from one year to the next, with annual maxima per marine area often greater than 1 million cells per liter, sometimes 10 million, in all provinces. Blooms over 1 million were observed from March to November in the Channel and Atlantic, all year round in the Mediterranean. The national record was reached in 2010 in the western Mediterranean with 60 million cells per liter. Several *Pseudo-nitzschia* species occurred in France. The species *P. australis* and *P. pseudodelicatissima* have been formally identified as the cause of AST episodes (Amzil *et al.*, 2001, Nézan *et al.*, 2006). It should also be noted that the following toxigenic species were present: *P. delicatissima*, *P. fraudulenta*, *P. multiseries*, *P. multistriata*, *P. pungens* (Lassus *et al.*, 2016). Given the difficulty of identifying species of this genus under the light microscope, the identifications in the REPHY data were mostly related to species groups called "complex": for example the *delicatissima* / *pseudodelicatissima* complex, the *seriata* complex, etc. It was therefore not possible to quantify the percentage of the contribution of toxic species in blooms compared to that of non-toxic species. However, some groups contain more toxic species, and the alert thresholds defined in the REPHY for triggering AST analysis takes this into account. The REPHY and REPHYTOX data showed that shellfish only became toxic after the development of at least several 100,000 cells per liter. Husson *et al.* (2016) used the REPHY data to characterize the seasonal dynamics and interannual variability of *Pseudo-nitzschia* blooms associated with scallop (*Pecten maximus*) contamination in six Channel and Atlantic coastal areas. They have shown that: (i) the extent and characteristics of blooms, as well as the frequency of associated toxicity episodes, varied widely by area; (ii) contamination of shellfish by AST was always preceded by significant blooms; (iii) irradiance and temperature played a major role in the initiation of blooms in the spring; (iv) AST episodes tended to be more frequent in bays strongly influenced by nutrient inputs.

Table 1 and Fig. 2 consider the two *Dinophysis* + *Phalacroma* genera together in the TU of the same name. Data from the three provinces for this TU were almost exclusively *Dinophysis* species. Otherwise, some *Phalacroma* species may also be toxic (Lassus *et al.*, 2016). The geographical distribution of these two genera together provided therefore relevant information in terms of risk. If this TU was present in all provinces, the concentrations were never high, or it was not present in all seasons, as evidenced by its weak dominance rank, especially in the Channel and Atlantic, respectively 57th and 58th (Table 1). The maximum annual concentrations were generally < 10,000 cells per liter, and were always < 100,000 cells per liter with the exception of Normandy, for which higher concentrations were recorded, e.g. 803,000 cells per liter on the coast of Calvados (south of Seine Bay) in 2006. If *Dinophysis* could be present in all seasons, it was generally observed from March-April in western and southern Brittany, and from July-August in Normandy. While it was rarely observed in winter on the Channel and Atlantic coasts, *Dinophysis* was present year round in the Mediterranean. In France, it was mainly *Dinophysis acuminata* and *D. sacculus* species that were responsible for DST episodes. But more episodic or more localized presence of the following potentially toxigenic species, could be observed: *D. acuta*, *D. caudata*, *D. fortii*, *D. norvegica, D. tripos*. Shellfish could become toxic even when *Dinophysis* was observed at very low concentration (sometimes < 100 cells per liter).

The dominance ranks of *Alexandrium* are very different according to the province (Table 1). It could be observed along the whole coastline, but showed a preference for certain areas (Fig. 2), in which the observed concentrations were high: on the north coast of Brittany, the annual maxima in the areas concerned often exceeded 1 million cells per liter, sometimes 10 million (always between June and August). The very regular presence of *Alexandrium* in Thau lagoon, all year round, but especially in autumn and winter, made it more dominant in the Mediterranean (29th) than on the other provinces. In France, the two toxic species that led to PST episodes were: *A. minutum* in the Channel and Atlantic (Lassus *et al.*, 2016);

A. pacificum in Thau lagoon (Masselin *et al.*, 2001; John *et al.*, 2014). REPHY and REPHYTOX data showed that shellfish became toxic only after significant development of a toxic species of *Alexandrium* (several thousand or tens of thousands of cells per liter, depending on regions and species), which explains why *Alexandrium* was an excellent indicator of future PST contamination. The areas most frequently affected by blooms greater than 100,000 cells per liter were located in northern and western Brittany, as well as in Thau lagoon. The maximum concentration recorded since 1999 was 41 million cells per liter in Brest harbor in 2012, and significant blooms have been recorded in this area since (Chapelle *et al.*, 2015). Guallar *et al.* (2017) showed that temperature was a key factor for bloom initiation, and river flow for the maximum abundance. However, this second factor was local, favoring growth through the supply of nutrients and limiting abundance by dilution. They also showed that *Alexandrium* has dynamic characteristics of an invasive species: an introduction period with low abundance, then a period with high abundance blooms and, finally, a period of presence but at lower abundance (because controlled by parasites or predators or competition). In the Mediterranean, the most prolific blooms of *A. pacificum* occurred in Thau lagoon. The fact that the strain of this species in this lagoon was related to a Japanese strain (Lilly *et al.*, 2002) has supported the hypothesis of an accidental introduction of *A. pacificum*. The cysts transported in the deballasting waters of Asian ships possibly contaminated the shellfish of the Spanish Mediterranean coast, leading to the introduction into Thau lagoon and the subsequent distribution of contaminated shellfish between France and Spain in the years 1980-1990. The *Alexandrium* blooms at Thau required special hydroclimatic conditions, combining temperature and wind factors (Laanaia *et al.*, 2013). The species *A. minutum* was also observed in another lagoon of western Mediterranean, Salses-Leucate, but generally at low concentrations.

While it does not appear to be a dominant TU (Table 1), *Karenia*, essentially represented in the data by *K. mikimotoi*, remained associated with significant harmful effects in France, by its hemolytic toxins acting directly on the marine fauna and flora, even if the events in question were not recent. The regular presence of *Karenia* was only found in certain areas, especially in western and southern Brittany, in low concentration. Over the period 1999-2018, the maximum observed was 136 million cells per liter for *K. mikimotoi*, in Brest harbor in 2002. In the years 1970-1980, *K. mikimotoi* was associated with harmful impacts on scallops (*Pecten maximus*): mortalities, growth inhibition, etc. in western Brittany (Lassus *et al.*, 2016). In June-July 1995, it caused massive mortalities of marine animals along the French Atlantic coast; this episode reached a considerable scale, never repeated since, by its geographical extension from western Brittany to south of the Loire river; the biological and economic impacts of these blooms were wide-ranging. It is worth remembering that the REPHY results were for near-shore waters, and could not be representative of phenomena that would have appeared offshore. This point is crucial for *K. mikimotoi*, whose near monospecific blooms were observed by satellite each year in July at the western entrance to the Channel (Ménesguen *et al.*, in revision), while Fig. 2 shows that the concentrations were not high in inshore coastal zones. The presence of *K. mikimotoi* offshore was particularly noted in the frontal zones and in the stratified waters where it could have access to high levels of ammonium (its preferred nitrogen nutrient form) resulting from the bacterial remineralization of the previous diatom blooms having sedimented at the bottom (Le Corre *et al.*, 1993, Ménesguen *et al.*, in revision). Worth noting is the presence in France, but rarely and always at low or very low concentration, of another species of *Karenia*, potentially producing brevetoxins: *K. papilionacea*. Because of the low concentrations observed, no harmful effect has ever been observed in France.

Another toxic species is worth mentioning: *Ostreopsis*, even if it does not appear in the first 40 dominant TU of Table 1. Present in the REPHY data since the 1990s, *Ostreopsis* has been particularly closely monitored since 2006, following respiratory disorders in bathers in the Marseille region (east Mediterranean). Only one species could be identified on a morphological and molecular basis, *O.* cf. *ovata* (Penna *et al.*, 2005), producer of palytoxins and ovatoxins, which release into the air in the form of aerosols can lead to irritation of the skin, eyes and respiratory system. *Ostreopsis* was observed almost every year in the French Mediterranean, particularly in more eastern areas not monitored by REPHY, where it regularly formed major blooms in summer (Amzil *et al.*, 2012; Lemée *et al,* 2012; Brissard *et al.*, 2014).

Among the toxic species of recent appearance in France are: *Karlodinium*, *Vulcanodinium rugosum* and *Azadinium*. It should be noted that not all of them are currently identified as such in the database, but most often under a higher level taxon (family, order, class, etc.), at least until a certain period of time. For example, *Karlodinium*, of which several species are known to be toxic to fish, has been present in the data only since 2008. However, fish mortalities observed in 1994 in Diana lagoon (Corsica), following a proliferation of *Gyrodinium corsicum*, known since as *Karlodinium corsicum* (Lassus *et al.*, 2016), proved that this genus was really present in French waters. *Vulcanodinium rugosum*, described by Nézan and Chomérat (2011), was present in the data from 2013 and identified as the source of pinnatoxin production in shellfish of Ingril lagoon on the Mediterranean west coast (Hess *et al.*, 2013). Its presence has been known since 1996, but it was only in 2009 with its massive proliferation at Ingril that it was possible to describe it. *Azadinium*, recognized as a producer of azaspiracids (Tillmann *et al.*, 2009) was present in the REPHY data from 2013 in the Atlantic. Azaspiracids were detected in France in small quantities as early as 2002 (Lassus *et al.*, 2016), then in 2008 (Amzil *et al.*, 2008): so, Azadinium was probably already present in France before 2013, but was not registered as such in the database.

Phaeocystis, ranked 2nd in dominance in the Channel (Table 1), was almost exclusively specific to this province, essentially along the northern part of the French coast, as shown in Fig. 2. It was present every year in this region, of which the coastal waters are among the most enriched in nutrients of the French coasts (Belin and Soudant, 2018). The annual maxima of *Phaeocystis* (which could be observed at all seasons) often exceeded 10 million cells per liter and almost always 1million, commonly forming spectacular foams. Blooms were also observed in the Bay of Mont-Saint-Michel (at the border between Normandy and Brittany): up to several million cells per liter in the 2000s.

At the 12th rank of dominance in the Atlantic (Table 1), *Lepidodinium* was mainly present in southern Brittany and around the Loire river estuary (Fig. 2). The annual maxima of

Lepidodinium often exceeded 10 million cells per liter, the record since 1999 being 100 million in northern Brittany in 2001. Blooms greater than 1 million cells per liter could be observed from March to November. The TU was entirely represented by the species *L. chlorophorum*, which colored the waters a very intense green. Although non-toxic, its proliferation regularly led however to significant anoxia, possibly resulting in mortalities of marine animals. The links between *L. chlorophorum* blooms and anoxia were described in Sournia *et al.* (1992), and could be found in REPHY data for more recent data: e.g. in Vilaine Bay (southern Brittany) in July 2018, 30 million cells per liter of *L. chlorophorum* were associated with three dissolved oxygen values below 2.9 mg/L, hypoxia threshold defined by Diaz and Rosenberg (2008). Green waters recorded on the Atlantic coast since 1982 could also be attributed retrospectively to this same species (Sournia *et al.*, 1992). Having a typically coastal distribution, its proliferation seemed to correlate with the river Loire inputs.

In addition to the results presented here, REPHY phytoplankton data sets (REPHY, 2017), have also been used to answer scientific questions, and have provided valuable information on phytoplankton biodiversity, spatial distribution and temporal variations of the various taxa along the French coastline (Beliaeff *et al.*, 2001; Gailhard *et al.*, 2002; 2003). More recently, David *et al.* (2012) analyzed the variability of phytoplankton groups in relation to climatic and environmental indices in the Bay of Biscay. Hernández-Fariñas *et al.* (2014) have shown modifications in species diversity over the last twenty years in the eastern Channel, in relation to hydroclimatic changes. Studies have also examined the conditions of the appearance of toxic species, e.g. Husson *et al.* (2016) for *Pseudo-nitzschia*, Chapelle *et al.* (2015) and Guallar *et al.* (2017) for *Alexandrium*. Hernández-Fariñas *et al.* (2015), analyzing the positions occupied by 35 phytoplankton taxa, have shown that several environmental variables could be considered as key factors controlling phytoplankton dynamics and influencing the structure of the community.

4.2 Toxins

The French coast has been regularly affected by the harmful effects of toxins for more than 30 years, the main problem being related to the contamination of bivalve molluscs, leading to bans on the fishing and sale of these shellfish during periods of variable duration.

4.2.1 History of toxic episodes in France

Shellfish farming is a historical activity in France for centuries. The two main shellfish exploited are oysters and mussels, with currently about 130,000 tons of oysters and about 65,000 tons of mussels produced each year, for a total production of about 200,000 tons all shellfish combined (http://coquillages.com /statistics/). These tonnages have been similar from year to year for decades (Buestel *et al.*, 2009). It should be noted that all French areas exploited by shellfish farmers or fishermen, in production areas, natural populations, and professional fishing zones, have always been monitored, except in two cases. The first case concerned scallop (*Pecten maximus*) fishing areas until 2004 for all categories of toxins; this has little impact on the interpretation of the evolution of the toxic episodes for DST and PST, the scallops being mainly concerned by AST. The second case concerned oysters (*Crassostrea gigas*) until 2001, which were not monitored as first-line for DST; given that DST data indicate that mussels have always been toxic prior to oysters over the 2001-2018 period, it is likely that the lack of oyster DST data prior to 2001 has a relatively small impact on overall interpretation DST data.

DST episodes have been regularly observed in all provinces since 1987 (Fig. 3). In fact, the first toxic episodes clearly associated with diarrheic toxins date back to 1983, when about 3,000 cases of diarrheic intoxication were reported in western France. But it is very likely that diarrheic toxins had contaminated shellfish long before that date: intoxications observed previously could have been confused with bacterial or viral poisoning (Caruana and Amzil, 2018). Lipophilic toxins incriminated in shellfish contamination were always OA (most often) or DTX (Amzil and Mathias, 2006; Amzil *et al.*, 2007; REPHYTOX, 2017). The REPHY and REPHYTOX data showed that these toxins were always present in association with the phytoplankton *Dinophysis*. Two other groups of lipophilic toxins were sometimes present in France, but always at very low concentration: AZA and YTX. Their presence could not be formally associated with their potential producers, although these were sometimes observed in water samples, respectively *Azadinium* for AZA, *Lingulodinium polyedrum* and *Protoceratium reticulatum* for YTX.

The first observation of PST dates from 1988 in northwestern Brittany, following the detection of significant concentrations of *Alexandrium minutum*. The Mediterranean province was affected ten years later, in 1998, in Thau lagoon. The presence of PST in France before 1988 is highly unlikely, given the following considerations: (i) the first observations of PST in Europe were made only in 1987 (Norway, Sweden, Denmark, Spain, Portugal) (information from: http://haedat.iode.org/index.php), (ii) in 1988, the monitoring was in place in France for 4 years, (iii) from 1988 to 1990, PST were observed only in the same two marine areas. The toxin profile of *A. minutum* was composed mainly of gonyautoxins-2,-3 (carbamate toxins) (Lassus *et al.*, 2004) and toxins-C1,-2 (N-sulfocarbamoyle toxins), while that of *A. pacificum* was composed mainly of gonyautoxins-1, -4, -5 and toxins C-2, C-4 (Masselin *et al.*, 2001). In the majority of cases, the presence of PST above the regulatory threshold was associated with significant developments of *Alexandrium*. The case of the Arcachon Bay episode in 2002-2003 remains the only one unsolved, since the presence of *Alexandrium* was not observed in the phytoplankton flora: no alternative explanation could be found for the presence of PST above the regulatory threshold, even if it was very low (864 µg STX equivalent per kg of shellfish flesh). With this exception, the *Alexandrium* / PST episode relationship cannot be called into question in the light of available 32-year data, and confirms the relevance of the PST surveillance strategy based on a phytoplankton indicator.

The first observation of AST dates back to 2000 in western Brittany (Amzil *et al.*, 2001; Nezan *et al.*, 2006). These toxins have been monitored along the entire coast only since 1999. It is therefore conceivable that occurrences of AST have not been detected before this date, but this assumption is unlikely. Indeed, the first observations of AST in Europe were made in 1992 (Sweden), then in 1995 (Spain, Portugal) and 1998 (UK) (information from:

http://haedat.iode.org/index.php), showing slow progression of AST toxin occurrences across Europe. In addition, a study conducted by LeDoux et al. (1996) on several areas of the French coast showed that these toxins were then absent from those areas, at least in the mussels. Finally, the system of control of food poisoning by the French authorities would have highlighted a problem related to the contamination of shellfish, which was not the case. The increase in AST episodes recorded since 2004 in the Channel and the Atlantic (Fig. 3) clearly corresponds to the beginning of the obligation to monitor scallops (*Pecten maximus*) in the European context, since these were previously excluded from the toxin monitoring system in France. In the different AST episodes, the toxin profile was mainly composed of domoic acid, and its epidomic acid analogue in small amounts, both produced by the toxic species of *Pseudo-nitzschia*. REPHY and REPHYTOX data showed that AST episodes were always associated with the genus *Pseudo-nitzschia* in coastal areas for which phytoplankton sampling could be performed at a depth close to that of toxins. Based on the REPHY-REPHYTOX available data over 32 years, the *Pseudo-nitzschia* / AST episodes relationship confirms the relevance of the AST surveillance strategy based on a phytoplankton indicator. In offshore areas (mainly scallop fishing areas), the association between phytoplankton and AST could not be evidenced from REPHY-REPHYTOX data alone, phytoplankton sampling not being carried out on deep scallops beds. But various *in situ* studies have shown the correspondence between the accumulation of AST in shellfish and the proliferation of toxic *Pseudo-nitzschia* species (Husson *et al.*, 2016; Thorel *et al*., 2017).

4.2.2 Affected areas and shellfish

The geographical distribution of DST episodes (Fig. 4) highlights areas that were always spared (e.g. northern France and northern Brittany). This could partly be explained by a lower frequency of *Dinophysis* occurrences in these areas (REPHY, 2017). In northern Brittany in particular, the hydrodynamics would not favor *Dinophysis* accumulation. In regularly affected areas, the periods of toxicity were clearly different according to the region. In the Channel, toxic episodes were generally observed between July and October. In the

Atlantic, they were: (i) from May, or even March-April, in western and southern Brittany, until August, sometimes until October-November; (ii) generally from May to July-August between the Loire and Gironde rivers; (iii) between April and August in the Arcachon Bay. In the Mediterranean: (i) in the open sea zones, toxic episodes were generally spread between March and October; (ii) in the lagoons, they could be observed all year long. The shellfish contaminated during the presence of DST were very varied (Table 2), especially in the Atlantic because the diversity of exploited shellfish was much greater than on the other provinces. Mussels were systematically contaminated before other shellfish and often at higher concentrations. The periods and duration of shellfish contamination-decontamination of offshore beds were most often different from those of shellfish close to the coast. The results prior to 2010 being qualitative (bioassay), the different toxic episodes could not be compared in terms of toxin concentrations for this period. The highest toxin concentration over the period 2010-2018 (expressed in µg of OA equivalent per kg of shellfish flesh) was recorded in the Arcachon Bay in April 2012: 37,296 µg/kg for *Mytilus* sp.; in this zone, at the same time, the following shellfish also reached their record, with 11,755 µg/kg for *Cerastoderma edule* and 2,278 µg/kg for *Ruditapes philippinarum*. The maxima observed for other species were: 4,792 µg/kg for *Donax trunculus* in western Brittany in June 2013, 2,621 µg/kg for *Polititapes rhomboides* in southern Brittany in June 2010, 2,305 µg/kg for *Pecten maximus* off the eastern Channel coast in September 2014, 1,067 µg/kg for *Aequipecten opercularis* in Jersey-Guernesey (Channel) in July 2018, 1,041 µg/kg for *Crassostrea gigas* in Salses Leucate lagoon in December 2012. For *Spisula solida, Glycymeris glycymeris, Mimachlamys varia*, the maxima were less than 500 µg/kg. During this period 2010-2018, the maximum concentrations of azaspiracids and yessotoxins were both observed in southern Brittany mussels, respectively: 53 µg of AZA equivalent in June 2017 and 1720 µg of YTX equivalent in July 2018.

Only two areas were affected for at least 5 years by PST episodes over the period 2000-2018 (Fig. 4), and were still so in recent years. The first was Brest harbor for which the first PST episodes were recent (2012), and recurred very regularly, the last having taken place in

2017. The second was Thau lagoon in the Mediterranean, for which the PST episodes were common between 1998 and 2007, and occurred again in 2015, 2016 and 2017. The three areas of the north coast of Brittany were mostly affected in the 1990s, with some sporadic episodes in the 2000s in one or other of these areas. For other areas (Arcachon Bay and Mediterranean areas except Thau lagoon), there were only very isolated episodes, dating back at least 10 years. The periods of PST episodes were very different between the Brittany areas and those of the Mediterranean. In Brittany, PST episodes have always been observed between June and August-September. In Thau lagoon, the PST episodes were systematically observed between September and December. The shellfish contaminated during the presence of PST were always among the following (Table 2): mussels, oysters, cockles, clams. This was partly explained by the fact that the areas concerned only contained these shellfish. But the wide range of data acquired with the systematic search for PST during fishing periods on various offshore shellfish of the Channel and the Atlantic (*Pecten maximus*, *Aequipecten opercularis*, *Mimachlamys varia*, *Callista chione*, *Glycymeris glycymeris*, *Polititapes rhomb*oides, *Spisula*, *Venus verrucosa*, *Ostrea edulis*), attest that these shellfish have never been contaminated by PST, at least during fishing periods. The highest concentration of PST (expressed in µg STX equivalent per kg of shellfish flesh) over the period 1987-2018 was recorded in Brest harbor in July 2012: 11,664 µg/kg for *Mytilus* sp. The maxima observed for the other shellfish species were: 7,360 µg/kg for *Crassostrea gigas* in northern Brittany in August 2001, 5,740 µg/kg for *Ruditapes decussatus* in Thau lagoon in November 2001, 3,300 µg/kg for *Cerastoderma edule* in northern Brittany in June 1998.

The areas and shellfish most affected by AST episodes over the period 2000-2018 (Fig. 4 and Table 2), and which were still so in recent years, were: (i) in Normandy, exclusively scallops (*Pecten maximus*) off the Bay of Seine; (ii) in western and southern Brittany, mostly *Pecten maximus*, but also *Donax* or *Polititapes rhomboides*, and sometimes *Mytilus edulis*, *Crassostrea gigas*, *Callista chione*, *Aequipecten opercularis*. On the Atlantic coast, between the rivers Loire and Gironde, the year 2010 was an atypical year: while AST were observed for the first time in this region, the variety of contaminated shellfish (mussels, oysters, clams, scallops, etc.) has never been found in later episodes. The hypothesis of a link between these episodes and the storm Xynthia which passed over much of France at the end of February 2010 is very likely. The conjunction of the storm with high tidal coefficients did indeed lead to the flooding of many coastal areas to the south of the Loire estuary (Bertin *et al.*, 2012). The marine waters, which were then loaded with nutrients, were probably the trigger of *Pseudo-nitzschia* blooms (Nézan *et al.*, 2010; Husson *et al,* 2016), with concentrations never observed before or since 2010 in this region. On the Mediterranean coast, AST episodes affected *Mytilus galloprovincialis* or *Donax* from several areas of the west coast in the early 2000s; since 2006, no more AST episodes have been observed. If whole regions were spared the presence of AST, this is not explained by lower frequencies or concentrations of *Pseudo-nitzschia* in these areas; in fact the year-by-year maps of developments of this taxon show significant blooms every year along the entire French coast (Fig. 2). The most plausible explanation remains that these blooms were often composed of several *Pseudo-nitzschia* species, whose toxic potential might be very different. Almost all AST episodes were observed between March and May including in the Mediterranean, over the entire period 2000-2018, and for all shellfish apart from scallops. This information corroborates the observations relating to *Pseudo-nitzschia*, of which proliferations in high concentration began most often in March-April. Conversely, scallops (*Pecten maximus*) could be contaminated all year round in the Channel (Amzil *et al.*, 2009) or the Atlantic, with the fishing periods extending most often from October to March. The highest concentration of AST (expressed in µg DA equivalent per kg shellfish flesh) was recorded in Brest harbor in April 2014: 861,000 µg/kg for *Pecten maximus*. The maxima observed for the other shellfish species were: 221,000 µg/kg for *Mytilus* sp. in Brest harbor in April 2014, 133,000 µg/kg for *Donax trunculus* in western Brittany in April 2017, 123,000 µg/kg for *Ruditapes philippinarum* in southern Brittany in April 2010,110,000 µg/kg for *Crassostrea gigas* in Brest harbor in April 2014. For the other shellfish, the maxima were less than 100,000 µg/kg.

5 Conclusions

REPHY phytoplankton data contains gems of knowledge regarding the temporal and spatial distribution of species in terms of presence, abundance and biodiversity. The elementary data analysis used here has enabled us to pick only the low hanging fruits. Among them, dominant Taxonomic Units of the French coast during the last 20 years were *Chaetoceros*, *Skeletonema*, Cryptophyceae, *Leptocylindrus* and *Pseudo-nitzschia*. Occurrences and frequencies of occurrence of 6 toxic and harmful TU showed different spatial patterns, suggesting a mosaic of phytoplankton ecological niches and of coastal ecosystems. The long-term information gathered over the years enabled the investigation of abundance time series, unusual events and conditions of appearance of these species. The patterns of change of shellfish toxicity episodes over 32 years appeared as a growing trend on the Atlantic coast in the overall number of toxic episodes since 1987; the appearance of AST episodes and the beginning of the obligation to monitor scallops, especially since 2005, explains this upward trend. It should be noted that PST and AST were very probably absent from the French coasts, respectively before 1988 and 2000. In addition, even by roughly estimating the number of occurrences of DST before the 1980s, a projection over 40 or 50 years would undeniably show that the presence of toxins in shellfish became, in the years 1980-1990, a priority public health concern and a major issue for shellfish farmers and fishermen in France. It was to be feared that other toxins could also appear: For this reason, in parallel with the monitoring carried out under REPHY-REPHYTOX, a shellfish watch is also undertaken by Ifremer for toxic micro-algae and toxins known in other countries, but not yet listed in France. This complementary system should make it possible to tailor the official surveillance process to enable it to keep up with new events as far as possible. For 30 years, the REPHY-REPHYTOX networks have fulfilled their objectives of scientific observation, regulatory surveillance and health controls. Among the major challenges that remain to be faced are ensuring sustainability through continuous adaptation to the objectives and to the enhancement of measurement methods, and to developing analysis strategies to deal with the extensive and complex data they involve.

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7 References

Aminot, A., Kérouel, R., 2004. Hydrologie des écosystèmes marins. Paramètres et analyses, Editions Ifremer, France.

Aminot, A., Kérouel, R., 2007. Dosage automatique des nutriments dans les eaux marines. Méthodes en flux continu. Editions Ifremer, France.

Amzil, Z., Fresnel, J., Le Gal, D., Billard, C., 2001. Domoic acid accumulation in French shellfish in relation to toxic species of *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima*. Toxicon, 39(8), 1245-1251. doi:https://doi.org/10.1016/S0041-0101(01)00096-4.

Amzil, Z., Mathias, A., 2006. First report on detection of okadaic acid 7-O-acyl-ester derivatives (DTX-3) in French shellfish. In Molluscan Shellfish Safety (K. Henshilwood, B. Deegan, T. McMahon, C. Cusak, S. Keaveney, J. Silke, M. O'Cinneide, D. Lyons, P. Hess, eds.), 150-161.

Amzil, Z., Royer, F., Sibat, M., Fiant, L., Gelin, M., Le Gal, D., Françoise, S, 2009. First report on Amnesic and Diarrheic toxins detection in French scallops during 2004 – 2005 monitoring Surveys. In: Proceedings of the 6th ICMSS-2007. Burrow R., Greening G., Miles C., Seamer C., Simmons G. Van de Riet J. and Busby P. (Eds), 307-314.

Amzil, Z., Sibat, M., Chomerat, N., Grossel, H., Marco-Miralles, F., Lemee, R., Nezan, E., Sechet, V., 2012. Ovatoxin-a and Palytoxin Accumulation in Seafood in Relation to *Ostreopsis* cf. *ovata* Blooms on the French Mediterranean Coast. Mar. Drugs, 10, 477–496. doi:https://doi.org/10.3390/md10020477

Amzil, Z., Sibat, M., Royer, F., Masson, N., Abadie, E., 2007. Report on the first detection of pectenotoxin-2, spirolide-A and their derivatives in French shellfish. Mar. Drugs, 5, 168-179.

Amzil, Z., Sibat, M., Royer, F., Savar, V., 2008. First report on azaspiracid and yessotoxin groups detection in French shellfish. Toxicon, 52, 39-48.

Anderson, D.M., 2009. Approaches to monitoring, control and management of harmful algal blooms (HABs). Ocean Coast. Manag. 52, 342. doi:http://dx.doi.org/10.1016/j.ocecoaman.2009.04.006

Anderson, D., Glibert, P., Burkholder, J., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25(4), 704–726.

Anonymous, 2008. EU-Harmonised Standard Operating Procedure for determination of domoic acid in shellfish and finfish by RP-HPLC using UV detection, version 1. European Union Reference Laboratory for Marine Biotoxins (EU-RL-MB).

Anonymous, 2014. EURLMB Standard Operating Procedure for PSP toxins by Mouse Bioassay, version 1. European Union Reference Laboratory for Marine Biotoxins (EU-RL-MB).

Anonymous, 2015. EU-Harmonised Standard Operating Procedure for determination of Lipophilic marine biotoxins in molluscs by LC-MS/MS, version 5. European Union Reference Laboratory for Marine Biotoxins (EU-RL-MB).

Beliaeff, B., Gros, P., Belin, C., Raffin, B., Gailhard, I., Durbec, J.P., 2001. ‘Phytoplankton events’ in French coastal waters during 1987-1997. Oceanol. Acta, 24(5), 425-433. doi:https://doi.org/10.1016/S0399-1784(01)01156-2.

Belin, C., Soudant, D., 2018. Trente années d'observation des microalgues et des toxines d'algues sur le littoral. Editions QUAE, France. doi:https://archimer.ifremer.fr/doc/00478/58981/

Bertin, X., Bruneau, N., Breilh, J. F., Fortunato, A. B., Karpytchev, M., 2012. Importance of wave age and resonance in storm surges: The case Xynthia, Bay of Biscay. Ocean Modelling, 42, 16-30.

Brissard, C., Herrenknecht, C., Sechet, V., Herve, F., Pisapia, F., Harcouet, J., Lemee, R., Chomerat, N., Hess, P., Amzil Z., 2014. Complex Toxin Profile of French Mediterranean *Ostreopsis* cf. *ovata* Strains, Seafood Accumulation and Ovatoxins Prepurification. Mar. Drugs, 12(5), 2851-2876. doi:https://doi.org/10.3390/md12052851

Buestel, D., Ropert M., Prou J., Goulletquer P., 2009. History, Status, and Future of Oyster Culture in France. *Journal of Shellfish Research*, 28(4), 813-820. https://doi.org/10.2983/035.028.0410

Caruana, A.M.N., Amzil, Z., 2018. Microalgae and Toxins. In Microalgae in Health and Disease Prevention (I. Levine et J. Fleurence, eds.), chapter 13, 1-44.

Chapelle, A., Le Gac, M., Labry, C., Siano, R., Quere, J., Caradec, F., Le Bec, C., Nézan, E., Doner, A., Gouriou, J., 2015. The Bay of Brest (France), a new risky site for toxic *Alexandrium minutum* blooms and PSP shellfish contamination. Harmful Algae news, 51, 4-5. doi:http://archimer.ifremer.fr/doc/00278/38921/

David, V., Ryckaert, M., Karpytchev, M., Bacher, C., Arnaudeau, V., Vidal, N., Maurer, D., Niquil, N., 2012. Spatial and long-term changes in the functional and structural phytoplankton communities along the French Atlantic coast. Estuar. Coast.Shelf S., 108, 37-51. doi;https://doi.org/10.1016/j.ecss.2012.02.017

Devlin, M., Best, M., Bresnan, E., Scanlan, C., Baptie, M., 2014. Water Framework Directive: The development and status of phytoplankton tools for ecological assessment of coastal and transitional waters. United Kingdom.

Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science, 321(5891), 926-929. doi:https://doi.org/10.1126/science.1156401.

Gailhard, I., Durbec, J.P., Beliaeff, B., Sabatier, R., 2003. Phytoplankton ecology along French coasts: inter-site comparison. Comptes Rendus Biologies, 326(9), 853-863. doi:https://doi.org/10.1016/j.crvi.2003.09.002

Gailhard, I., Gros, P., Durbec, J.P., Beliaeff, B., Belin, C., Nézan, E., Lassus, P., 2002. Variability patterns of microphytoplankton communities along the French coasts. Mar. Ecol. Prog. Ser., 242, 39-50. doi:https://doi.org/10.3354/meps242039

Guallar, C., Bacher, C., Chapelle, A., 2017. Global and local factors driving the phenology of *Alexandrium minutum* (Halim) blooms and its toxicity. Harmful Algae, 67, 44-60. doi:http://doi.org/10.1016/j.hal.2017.05.005

Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase. Phycologia, 32(2), 79-99. doi:https://doi.org/10.2216/i0031-8884-32-2-79.1

Hernández-Fariñas, T., Bacher, C., Soudant, D., Belin, C., Barillé, L., 2015. Assessing phytoplankton realized niches using a French National Phytoplankton Monitoring Network. Estuar. Coast.Shelf S., 159, 15-27. doi:http://dx.doi.org/10.1016/j.ecss.2015.03.010

Hernández-Fariñas, T., Soudant, D., Barillé, L., Belin, C., Lefebvre, A., Bacher, C., 2014. Temporal changes in the phytoplankton community along the French coast of the eastern English Channel and the southern Bight of the North Sea. Ices J.Mar. Sci., 71(4), 821-833. doi:http://dx.doi.org/10.1093/icesjms/fst192

Hess, P., Abadie, E., Herve, F., Berteaux, T., Sechet, V., Araoz, R., Molgo, J., Zakarian, A., Sibat, M., Rundberget, T., Miles, C.O., Amzil, Z., 2013. Pinnatoxin G is responsible for atypical toxicity in mussels (*Mytilus galloprovincialis*) and clams (*Venerupis decussata*) from Ingril, a French Mediterranean lagoon. Toxicon, 75, 16-26. doi:http://doi.org/10.1016/j.toxicon.2013.05.001

Husson, B., Hernández-Fariñas, T., Le Gendre, R., Schapira, M., Chapelle, A., 2016. Two decades of *Pseudo-nitzschia* spp. blooms and king scallop (*Pecten maximus*) contamination by domoic acid along the French Atlantic and English Channel coasts: seasonal dynamics, spatial heterogeneity and interannual variability. Harmful Algae, 51, 26-39. doi:http://doi.org/10.1016/j.hal.2015.10.017

John, U., Litaker, R.W., Montresor, M., Murray, S., Brosnahan, M.L., Anderson, D.M., 2014. Formal Revision of the *Alexandrium tamarense* Species Complex (Dinophyceae) Taxonomy:

The Introduction of Five Species with Emphasis on Molecular-based (rDNA) Classification. Protist. 165: 779-804. https://doi.org/10.1016/j.protis.2014.10.001

Koslow, J.A., Couture, J., 2013. Follow the Fish. A global, long-term programme of ecological monitoring is needed to track ocean health,. Nature, 502(7470), 163-164. doi:https://doi.org/10.1038/502163a

Laanaia, N., Vaquer, A., Fiandrino, A., Genovesi, B., Pastoureaud, A., Cecchi, P., Collos, Y., 2013. Wind and temperature controls on *Alexandrium* blooms (2000-2007) in Thau lagoon (Western Mediterranean). Harmful Algae, 28, 31-36. doi:https://doi.org/10.1016/j.hal.2013.05.016

Lassus, P., Baron, R., Garen, P., Truquet, P., Masselin, P., Bardouil, M., Leguay, D., Amzil, Z., 2004. Paralytic shellfish poison outbreaks in the Penze estuary: Environmental factors affecting toxin uptake in the oyster, *Crassostrea gigas*. Aquat. Living Resour., 17(2), 207-214

Lassus, P., Chomérat, N., Hess, P., Nézan, E., 2016. Toxic and Harmful Micro-algae of the World Ocean / Micro-algues toxiques et nuisibles de l'océan mondial. Denmark, International Society for the Study of Harmful Algae / Intergovernmental Oceanographic Commission of Unesco. IOC Manuals and Guides, 68 (Bilingual English/French).

Le Corre, P., L'Helguen, S., Wafar, M., 1993. Nitrogen source for uptake by *Gyrodinium* cf.*aureolum* in a tidal front. Limnol. Oceanogr., 38(2), 446-451. doi:http://www.jstor.org/stable/2837825

LeDoux, M., Belin, C., Lotfi, Y., Lassus, P., Fremy, J.M., 1996. Domoic acid: State of contamination of shellfish in France - Preliminary study. In Proceedings of the Seventh International Conference on Toxic Phytoplankton (T. Yasumoto, Y. Oshima, Y. Fukuyo, eds), IOC of Unesco, 135-142.

Lemee, R., Mangialajo, L., Cohu, S., Amzil, Z., Blanfune, A., Chomerat, N., Ganzin, N., Gasparini, S., Grossel, H., Guidi-Guivard, L., Hoareau, L., Le Duff, F., Marro, S., Simon, N., Nézan, E., Pedrotti, M.L., Sechet, V., Soliveres, O., Thibaut, T., 2012. Interactions between scientists, managers and policy makers in the framework of the French MediOs project on *Ostreopsis* (2008-2010). Cryptogamie Algol., 33(2), 137-142.

Leterme, S.C., Pingree, R.D., Skogen, M.D., Seuront, L., Reid, P.C., Attrill, M.J., 2008. Decadal fluctuations in North Atlantic water inflow in the North Sea between 1958-2003: impacts on temperature and phytoplankton populations. Oceanologia, 50(1), 59-72.

Lilly, E.L., Kulis, D.M., Gentien, P., Anderson, D.M., 2002. Paralytic shellfish poisoning toxins in France linked to a human-introduced strain of *Alexandrium catenella* from the western Pacific: evidence from DNA and toxin analysis. J. Plankton Res., 24(5), 443-452.

Masselin, P., Amzil, Z., Abadie, E., Nézan, E., Le Bec, C., Chiantella, C., Truquet, P., 2001. Paralytic shellfish poisoning on the French Mediterranean coast in the autumn 1998 : *Alexandrium tamarense* complex (Dinophyceae) as causative agent. In Harmful Algal Blooms 2000 (G.M. Hallegraeff, S.I. Blackburn, C.J. Bolch, R.J. Lewis, eds.), JOC of Unesco Publish., 26-29.

Ménesguen, A., Dussauze, M., Thouvenin, B., in revision. Ecological model of the Bay of Biscay and English Channel shelf for ecological status assessment. Part. 2: Three types of HAB (*Karenia*, *Phaeocystis*, *Pseudo-nitzschia*) and their link with nitrogen anthropogenic enrichment. Ocean Modelling.

Nézan, E., Antoine, E., Fiant, L., Amzil, Z., Billard, C., 2006. Identification of *Pseudo-nitzschia australis* and *P. multiseries* in the bay of Seine. Was there a relation to presence of domoic acid in king scallops in autumn 2004? Harmful Algae News, 31, 1-3.

Nézan, E., Chomérat, N., 2011. *Vulcanodinium rugosum* gen. et sp. nov. (Dinophyceae), un nouveau dinoflagellé marin de la côte méditerranéenne française. Cryptogamie Algol., 32(1), 3-18.

Nézan, E., Chomérat, N., Bilien, G., Boulben, S., Duval, A., Ryckaert, M., 2010. *Pseudo-nitzschia australis* on French Atlantic coast - an unusual toxic bloom. Harmful Algae News 41, 1-2.

Penna, A., Vila, M., Fraga, S., Giacobbe, M.G., Andreoni, F., Riobo, P., Vernesi, C., 2005. Characterization of *Ostreopsis* and *Coolia* (Dinophyceae) isolates in the western Mediterranean Sea based on morphology, toxicity and internal transcribed spacer 5.8s rDNA sequences. J. Phycol. 41, 212–225. doi:https://doi.org/10.1111/j.1529-8817.2005.04011.x

Philippart, C.J.M., Cadée, G.C., Van Raaphorst, W., Riegman, R., 2000. Long-term phytoplankton-nutrient interactions in a shallow coastal sea: Algal community structure, nutrient budgets, and denitrification potential. Limnol. Oceanogr., 45(1), 131-144. doi:https://doi.org/10.4319/lo.2000.45.1.0131

QGIS, 2018. Système d'information géographique QGIS. Open Source Geospatial Foundation Project. doi:https://qgis.org/fr/site/

R Development Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

REPHY, 2017. REPHY dataset. French Observation and Monitoring program for Phytoplankton and Hydrology in coastal waters. 1987-2016 Metropolitan data. SEANOE. doi:http://doi.org/10.17882/47248

REPHYTOX, 2017. REPHYTOX dataset. French Monitoring program for Phycotoxins in marine organisms. Data since 1987. SEANOE. doi:http://doi.org/10.17882/47251

Sanders, H.L. 1960. Benthic studies in Buzzards Bay. III. The structure of the soft-bottom community. Limnol. Oceanogr., 5, 138-153. doi:https://doi.org/10.4319/lo.1960.5.2.0138

Sournia, A., Belin, C., Billard, C., Catherine, M., Erard-Le Denn, E., Fresnel, J., Lassus, P., Pastoureaud, A., Soulard, R., 1992. The repetitive and expanding occurrence of a green, bloom-forming dinoflagellate (Dinophyceae) on the coasts of France. Cryptogamie Algol., 13(1), 1-13. doi:http://archimer.ifremer.fr/doc/00133/24470/

Thorel, M., Claquin, P., Schapira, M., Le Gendre, R., Riou, P., Goux, D., Le Roy, B., Raimbault, V., Deton-Cabanillas, A.F, Bazin, P., Kientz-Bouchart, V., Fauchot, J., 2017. Nutrient ratios influence variability in *Pseudo-nitzschia* species diversity and particulate domoic acid production in the Bay of Seine (France). Harmful Algae, 68, 192-205. doi:10.1016/j.hal.2017.07.005

Tillmann, U., Elbrachter, M., Krock, B., John, U., Cembella, A., 2009. *Azadinium spinosum* gen. et sp. nov. (Dinophyceae) identified as a primary producer of azaspiracid toxins. Eur. J. Phycol., 44(1), 63-79.

Utermöhl, von H., 1931. Neue Wege in der quantitativen Erfassung des Planktons (Mit besondere Beriicksichtigung des Ultraplanktons). Ver. Int. Verein. Theor. Angew. Limnol., 5, 567-595.

Vaquer, A., Troussellier, M., Courties, C., Bibent, B., 1996. Standing stock and dynamics of picophytoplankton in the Thau lagoon (northwest Mediterranean coast). Limnol. Oceanogr., 41(8), 1821-1828.

Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S.O., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D.M., Cochlan, W.P., 2015. Harmful algal blooms and climate change: learning from the past and present to forecast the future. Harmful Algae 49, 68–93. doi:http://dx.doi.org/10.1016/j. hal.2015.07.009

Yasumoto, T., Murata, M., Oshima, Y., Matsumoto, G.K., Clardy J., 1984. Diarrhetic Shellfish Poisoning. *In*: Sea Food Toxins, Ragelis E.P. (ed). ACS symposium series, n°262, 208-214. doi:10.1021/bk-1984-0262.ch019

Fig. 1

Fig. 1. The sampling stations of the REPHY network, classified according their belonging to the three components: Observation (36 stations), Surveillance (116 stations), strictly Health (71 stations).

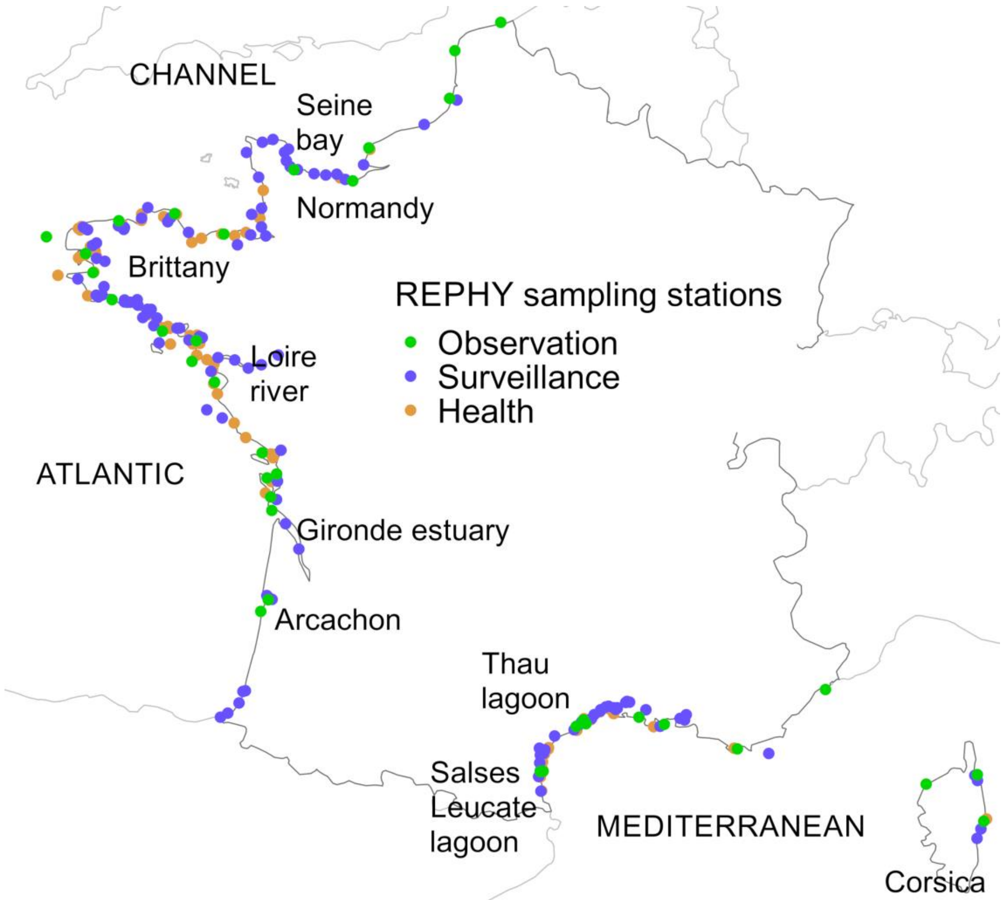

Table 1

Taxonomic Units (TU) by rank of dominance (calculated by a method adapted from the Sanders index), over the period 1999–2018, in descending order over the entire French coastline (column All). Ranks are also indicated for each province (Ch = Channel, Atl = Atlantic, Med = Mediterranean). Only TU ranked in the top 40 ranks from one or other province are shown in the table. 'n.o.' = not observed. Important details: most TU are groups of genera and / or species. For the others, there are several cases identified by different colors:

	By geographic region			
	All	Ch	Atl	Med
Chaetoceros	1	1	3	1
Skeletonema	2	3	1	4
Cryptophyceae	3	6	4	3
Leptocylindrus	4	8	2	5
Pseudo-nitzschia	5	9	5	2
Thalassiosira + *Porosira*	6	5	6	22
Guinardia	7	4	11	19
Cylindrotheca + …. (1)	8	12	8	6
Paralia	9	7	7	41
Phaeocystis	10	2	64	n.o.
Asterionella + *Asterionellopsis* + *Asteroplanus*	11	11	9	12
Other Gymnodiniaceae	12	16	10	8
Prorocentrum	13	25	13	7
Thalassionema + *Thalassiothrix* + *Lioloma*	14	13	20	9
Neocalyptrella + ….(2)	15	10	15	16
Navicula + *Fallacia* + *Haslea* + *Lyrella* + *Petroneis*	16	17	17	11
Scrippsiella + *Ensiculifera* + *Pentapharsodinium*	17	28	14	10
Dactyliosolen	18	14	24	14
Other (Cymatosiraceae + Plagiogrammaceae)	19	15	18	86
Protoperidinium + *Peridinium*	20	29	21	17
Other (Euglenoidea + …) (3)	21	22	27	20
Lepidodinium	22	65	12	n.o.
Ciliophora	23	18	22	n.o.
Cerataulina	24	26	19	28
Dictyocha + *Octactis*	25	39	30	18
Other Fragilariaceae + Toxariaceae	26	47	16	115
Licmophora	27	54	63	13
Pleurosigma + *Gyrosigma*	28	30	28	23
Cocconeis	29	91	72	15
Heterocapsa	30	35	34	24
"Pennate" diatoms	31	21	33	34
Melosira	32	37	23	49
Ceratium + *Neoceratium* + *Tripos*	33	81	41	21
Biddulphia + … (4)	34	20	38	58
Lauderia + *Detonula*	35	23	35	47
Rhaphoneis + *Delphineis*	36	19	56	n.o.
"Centric" diatoms	37	38	26	78
Ditylum	38	27	32	54

	By geographic region			
	All	Ch	Atl	Med
Other Chaetocerotaceae	39	n.o.	25	n.o.
Katodinium	40	40	42	32
Other Thalassionemataceae	41	43	36	33
Coscinodiscus + *Stellarima*	42	45	29	60
Chromista incertae sedis	43	24	59	n.o.
Other Thalassiosiraceae	44	32	37	n.o.
Grammatophora	45	53	47	30
Euglenaceae + … (5)	46	36	45	50
Dinophysis + *Phalacroma*	47	57	58	25
Other Peridiniales	48	52	31	95
Alexandrium	49	48	66	29
Other Bacillariaceae	50	34	48	116
Eucampia + *Climacodium*	51	31	52	62
Oxytoxum + *Corythodinium*	52	131	103	26
Diploneis	53	50	70	31
Bacteriastrum	54	83	54	35
Actinocyclus	55	108	160	27
Other Cymatosiraceae	56	105	39	n.o.
Bacillaria	57	42	51	65
"Phytoflagellates except dinoflagellates"	58	79	40	n.o.
Lithodesmium	59	69	43	108
Torodinium	60	56	55	46
Karenia	61	63	65	37
Scenedesmus	62	49	57	53
Diatoma + *Fragilaria*	63	76	46	71
Other Entomoneidaceae	64	n.o.	44	n.o.
Gonyaulax + *Protoceratium*	65	60	67	42
Synedra + *Toxarium*	66	74	49	56
Mesodinium	67	51	53	74
Dinobryon	68	72	50	61
Diplopsalis + … (6)	69	66	76	40
Amphidinium	70	55	77	45
Other Chlorophyceae	71	33	110	80
Hemiaulus	72	110	102	36
Lingulodinium	73	126	123	38
Other Ceratiaceae	74	41	88	91
Other Naviculales	75	n.o.	n.o.	39

(1) *Cylindrotheca* + *Ceratoneis* + *Nitzschia* + *Hantzschia*
(2) *Neocalyptrella* + *Proboscia* + *Rhizosolenia* + *Pseudosolenia*
(3) Euglenoidea + Euglenia + Euglenida + Eutreptiida + Eutreptiales
(4) *Biddulphia* + *Odontella* + *Trigonium* + *Trieres* + *Isthmia*
(5) Euglenaceae + *Euglena* + Eutreptiaceae + *Eutreptia* + *Eutreptiella*
(6) *Diplopsalis* + *Diplopelta* + *Diplopsalopsis* + *Preperidinium* + *Oblea*

1st to 10th rank
11th to 20th rank
21st to 30th rank
31st to 40th rank

Fig. 2

Maps showing six TU among the toxic or harmful species. Each map summarizes the results for a TU over 20 years (1999–2018), by marine area. The results are presented by concentration range, in cells per liter. The size of the symbols is proportional to the P90 of the cell concentration. Transparency of color disks varies linearly with the number of TU occurrences, from transparent (i.e. one occurrence) to opaque (i.e. 240 occurrences, that is, identified each month during 20 years).

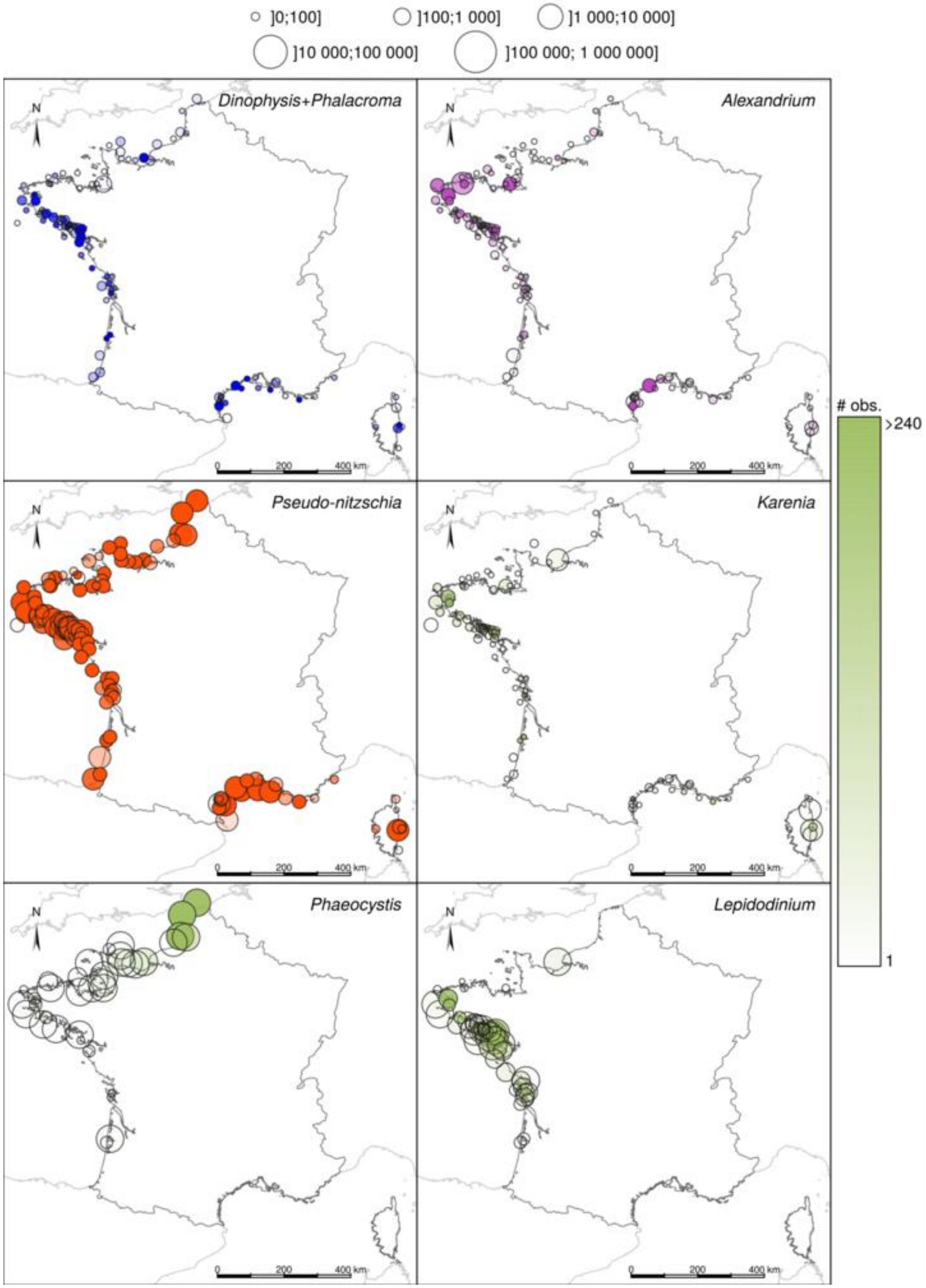

Fig. 3

Percentage of marine areas affected by a toxic episode, for each province (Channel, Atlantic, Mediterranean), relative to the total number of marine areas in each of these provinces (38 areas for Channel, 54 for Atlantic, 31 for Mediterranean). Per year from 1987 to 2018, and for each category of toxins. If two categories of toxins have affected the same area in the same year, each is counted, which increases the total percentage by the same amount.

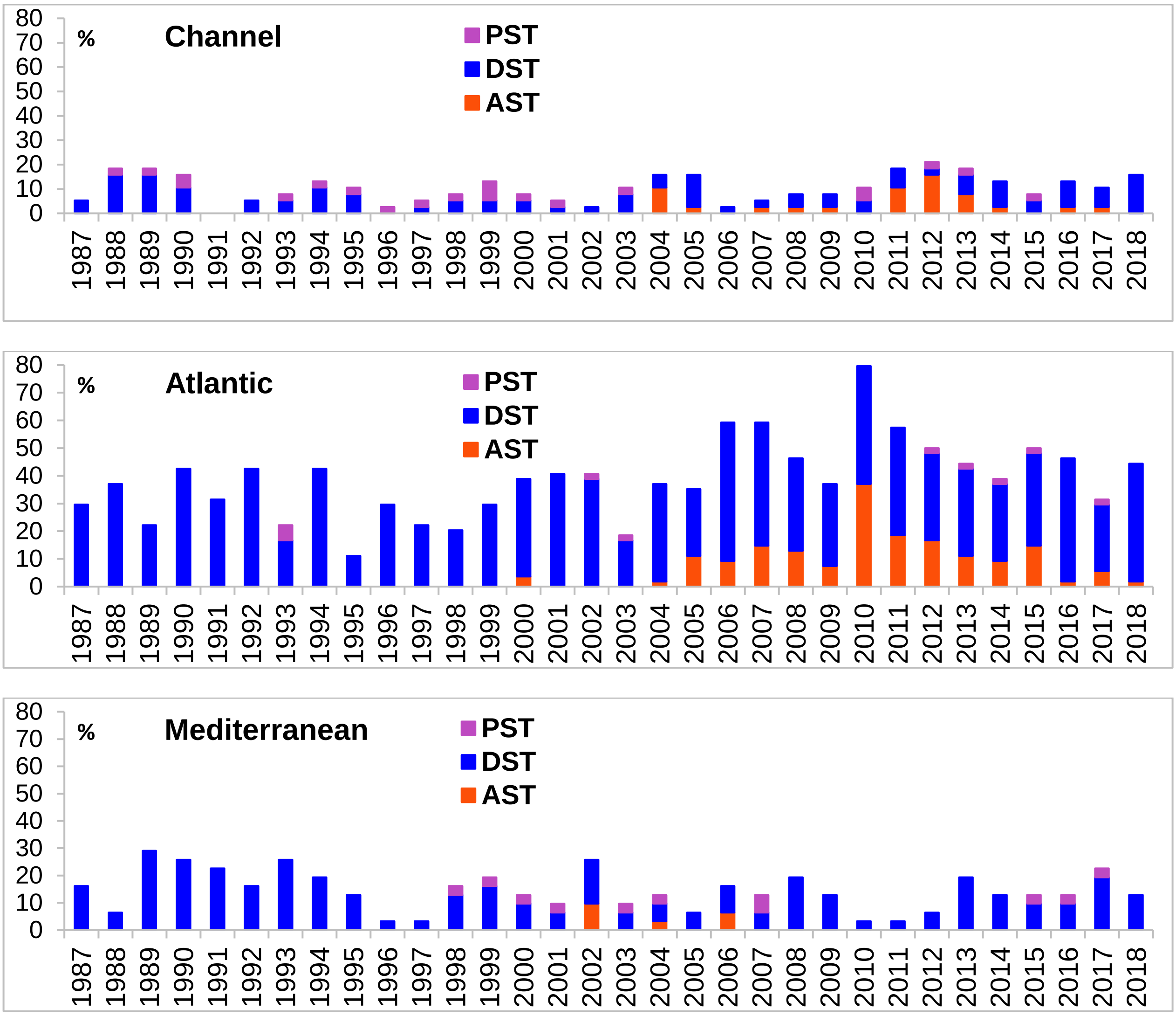

Fig. 4

Maps of toxic episodes over the period 2000–2018, for each category of toxins (DST, PST, AST); in number of years affected by at least one toxic episode in the year, for each marine area.

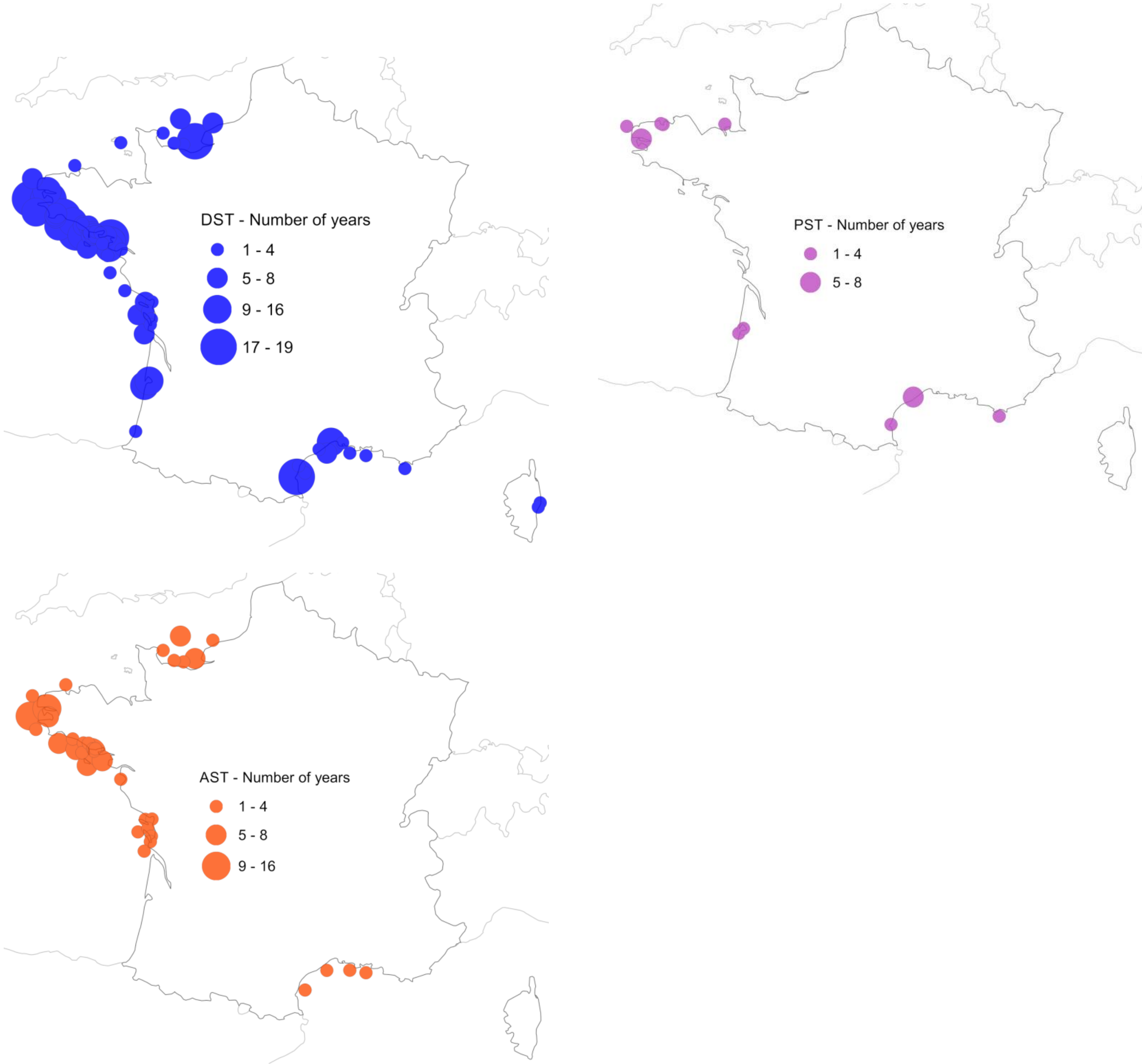

Table 2

Number of years for which shellfish have been affected by a toxic episode, in at least one marine area of the province. By shellfish, category of toxins and province. Since 1987 for DST, 1988 for PST, 2000 for AST, and until 2018, inclusive.

Toxin	DST (since 1987)			PST (since 1988)			AST (since 2000)		
Maritime province	Ch.	Atl.	Med.	Ch.	Atl.	Med.	Ch.	Atl.	Med.
Mytilus	30	32	32	12	8	10		5	2
Crassostrea gigas	1	17	16	10	2	5		2	
Donax	5	32	11					9	2
Cerastoderma edule		23		4					
Ruditapes		10	6			2		1	
Pecten maximus	9	6					11	15	
Aequipecten opercularis	3	3						1	
Mimachlamys		2							
Polititapes rhomboides		20						2	
Glycymeris glycymeris		12						1	
Callista chione		2						1	
Spisula		5							