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1 Three decades of data on phytoplankton and phycotoxins on the French coast:

- 2 lessons from REPHY and REPHYTOX.
- 3

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9

10 Abstract

11 In France, REPHY (Observation and Surveillance Network for Phytoplankton and Hydrology 12 in coastal waters) and REPHYTOX (Monitoring Network for Phycotoxins in marine 13 organisms) have been contributing to long-term time series on ocean health for more than 30 14 years. The aim of this paper is to describe these networks and to highlight their key results. 15 Over the last 20 years, phytoplankton flora analysis on French coasts from the Channel to 16 Mediterranean has shown that the five "emblematic" taxa are *Chaetoceros*, *Skeletonema*, 17 Cryptophyceae, Leptocylindrus and Pseudo-nitzschia. The latter, together with the taxa of 18 interest Dinophysis + Phalacroma, Alexandrium, and Karenia, have been consistently 19 recorded along the entire French coastline. However, when taking into account frequency of 20 occurrence some taxa exhibit more distinct geographical distributions. In particular, the 21 occurrence of *Phaeocystis* appeared to be strongly specific to the northern coasts of the 22 Channel. French coasts have been regularly affected since the 1980s by the presence of 23 toxins in bivalve molluscs, leading to bans on fishing and sale of shellfish during periods of 24 varying duration. Three categories of toxins were involved. PST and AST were absent from 25 the French coasts, respectively before 1988 and 2000. DST (Diarrheic Shellfish Toxins) have 26 affected many areas along the whole coast every year since 1987. For PST (Paralytic 27 Shellfish Toxins), only a few areas have been affected, sometimes sporadically, since 1988 in 28 the Channel, 1993 in the Atlantic, and 1998 in the Mediterranean. Many areas have been

- 29 impacted since 2000 by AST (Amnesic Shellfish Toxins) episodes, mainly affecting scallops
- 30 in the Channel and on Atlantic coasts. The patterns of change of shellfish toxicity episodes
- 31 showed no real trend in any province over the entire period 1987-2018.
- 32

33 Keywords

- 34 Monitoring network; Phytoplankton time-series; Dominant species; DST-Diarrheic Shellfish
- 35 Toxins; PST-Paralytic Shellfish Toxins; AST-Amnesic Shellfish Toxins
- 36

37 Terminology

38 Taxonomic Units (TU): taxa clusters, most often groups of genera and / or groups of species,

39 described to allow data processing.

40 Dominant taxa: the taxa most present on the French coast, calculated by a method derived41 from the Sanders index (1960).

42 Emblematic taxa: some of the most dominant taxa + some toxic or harmful taxa that are not
43 dominant. These taxa are considered emblematic either on the whole French coast or in one

44 of the three provinces (Channel, Atlantic, Mediterranean).

45

46 **1** Introduction

47 Phytoplankton provide key ecosystem services in terms of the functioning of the biological 48 pump, regulation of climate, generating half of the oxygen in our atmosphere, and as the 49 base of marine food webs. Numerous global changes since the beginning of the industrial 50 era, such as eutrophication, warming, acidification and deoxygenation of the ocean, 51 represent key stressors for marine ecosystems. Phytoplankton responses to these include: 52 (i) increase in phytoplankton biomass and oxygen depletion in bottom waters (Diaz and 53 Rosenberg, 2008); (ii) changes in structure of phytoplankton communities in response to 54 changes in nitrogen / phosphorus / silica ratios (Philippart et al., 2000); (iii) increased 55 frequency of harmful algal blooms (Hallegraeff, 1993; Anderson et al., 2002; Anderson, 2009; 56 Wells et al. 2015); (iv) alteration of the phenology of phytoplankton communities (Leterme et

57 al., 2008). Human health can be impacted by species that produce toxins that are dangerous 58 for humans who consume seafood, as well as environmental impacts from species that are 59 toxic or harmful for the marine fauna and flora. For all these reasons, knowledge of 60 phytoplankton dynamics is essential. Most natural ocean processes vary on annual to 61 decadal timescales and to identify shifts resulting from climate change, observations need to 62 span at least 30 or preferably 50 years or more (Koslow and Couture, 2013). In this context, 63 the acquisition of long-term series of phytoplankton species has become a priority in the 64 monitoring of the marine environment, knowing that long-term series on phytoplankton are 65 not common worldwide. The study of these issues is already benefiting from the availability of 66 long-term time series from coastal sites subject to contrasting environmental and human 67 pressures at a global scale. For example: (i) plankton observation data produced since 1931 68 by the Continuous Plankton Recorder (CPR), initially deployed in the North Sea and then 69 extended to the North-East Atlantic and now globally, (ii) the data series since 1962 on 70 plankton 'Helgoland Roads', located in a German archipelago in the southeast of the North 71 Sea.

72

73 In France, the creation in 1984 of the monitoring network REPHY-REPHYTOX by Ifremer 74 was part of this process. The objective of REPHY is to acquire data of the biomass, 75 abundance and composition of marine phytoplankton in coastal and lagoon waters, as well 76 as the associated hydrological parameters. These data cover in particular the spatio-77 temporal distribution of phytoplankton species, and the identification of exceptional blooms. 78 The objective of REPHYTOX is the detection and monitoring of the three categories of toxins 79 that can accumulate in bivalve molluscs, which are regulated at European level: lipophilic 80 toxins (including DST), PST and AST, which are respectively responsible for the following 81 human syndromes: DSP (Diarrheic Shellfish Poisoning), PSP (Paralytic Shellfish Poisoning) 82 and ASP (Amnesic Shellfish Poisoning). The two networks are closely associated, since the 83 monitoring of toxic phytoplankton by REPHY is used for triggering toxin analysis by 84 REPHYTOX and for a better understanding of shellfish contamination. REPHY is divided into

85 three components: Observation, Surveillance and Health (Belin and Soudant, 2018). The aim 86 of the Observation component is to seek answers to research questions such as: (i) what are 87 the responses of phytoplankton communities to environmental changes (e.g. Hernández-88 Fariñas et al., 2014); (ii) participate in the definition of phytoplankton ecological niches 89 (Hernández-Fariñas et al., 2015); (iii) detect variations in phenology (Guallar et al., 2017); (iv) 90 participate in the characterization of traits and functional groups (David et al., 2012). The 91 main aim of the Surveillance component is to participate, in addition to the Observation 92 component, in the assessment of the quality of the coastal waters through indicators, in 93 particular those described that meet the requirements of the European directives WFD 94 (Water Framework Directive) and MSFD (Marine Strategy Framework Directive). The 95 purpose of the Health component is to participate in the triggering of shellfish sampling 96 carried out within the framework of REPHYTOX network, by supplementing the results 97 already acquired on the toxic species by the two other components. REPHYTOX monitoring 98 applies to shellfish harvested for professional purposes in their natural environment i.e. in 99 production areas and natural populations, and in professional fishing zones. In addition to 100 providing the necessary information to the authorities responsible for managing the public 101 health risk, this monitoring system makes it possible to acquire scientifically interesting data. 102

The purpose of this article is to present a comprehensive review of the data acquired since 104 1987: (i) on the phytoplankton of the French coast, with respect to the most dominant taxa 105 and a survey of the geographical distribution of some toxic or harmful species; (ii) on the 106 three categories of phycotoxins most widespread in France, with the temporal patterns of 107 change of episodes of toxicity, and their geographical distribution. Data presented here do 108 not include those from the French overseas departments/areas.

109

110 2 Material and methods

111 The REPHY and REPHYTOX monitoring networks were set up by Ifremer in 1984, with the112 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.

119

120 2.1 Sampling strategies

121 REPHY and REPHYTOX spread over the whole French coastline. The three marine 122 provinces of France are: the Atlantic Ocean, the Channel and the Mediterranean (see Fig. 1). 123 The Channel is an epicontinental sea of the Atlantic Ocean, located in North-West Europe 124 and separating France from the United Kingdom; the Eastern Channel is one of the busiest 125 maritime areas of the globe; the water of this zone is, because of the currents among most 126 important in the world, very turbid, while remaining oxygenated. The Mediterranean Sea is an 127 almost entirely closed intercontinental sea, bordered by the coasts of Southern Europe, 128 North Africa and West Asia; its opening to the Atlantic Ocean by the Strait of Gibraltar is 14 129 kilometers wide.

130 For REPHY, the Observation component comprised 36 sampling stations (Fig. 1). The 131 Surveillance component complemented this system with another 116 stations distributed in 132 water bodies not covered by the Observation component. The Health component included 71 133 additional stations with strictly health objectives, but also used data from 41 stations related 134 to the Observation or Surveillance components. The sampling strategy consisted of *in situ* 135 measurements, and water samples for microscopic analysis of phytoplankton and for 136 selected additional analyses. Sampling and measurements were carried out under specific 137 conditions, particularly at 1 m below the surface (dissolved oxygen was also measured at the 138 bottom) and within 2 hrs either side of high tide in the Channel and the Atlantic. The sampling 139 periods and frequencies, as well as the parameters to be measured, were different 140 depending on the REPHY component, and the type of water body. For REPHY Observation,

141 sampling was done all year round, once a fortnight. The mandatory parameters were: in situ 142 measurements (temperature, salinity, turbidity, dissolved oxygen), microscopic analysis of all 143 phytoplankton ('total flora'), chlorophyll a, pheopigments and nutrients. Dissolved oxygen was 144 only measured in Summer from June to September. Phytoplankton pigments were also 145 analyzed on samples from a selection of stations spread along the whole coastline. For 146 REPHY Surveillance, sampling was generally done all year round, once a month. For most 147 stations, the mandatory parameters were: in situ measurements, microscopic analysis of a 148 selection of phytoplankton species ('targeted flora'), chlorophyll a, pheopigments and 149 nutrients. However, some analyses were limited to one period of the year: (i) for Channel and 150 Atlantic coasts, chlorophyll a and nutrients were measured respectively from March to 151 October, and from November to February, (ii) dissolved oxygen was measured from June to 152 September. Otherwise, for a few stations in Channel or Atlantic transitional water bodies, only 153 in situ measurements and nutrient analysis were performed. In the Mediterranean lagoons, 154 an estimation of nano- and pico-phytoplankton by flow cytometry was made in addition, from 155 June to August. For REPHY Health, the sampling period at strictly health-related stations was 156 dependent on the context: all year round, or over a limited period. The frequency was once a 157 week. The only mandatory parameter was the microscopic identification of toxic 158 phytoplankton species ('toxic flora'). At the Observation and Surveillance components 159 stations identified as being useful for the Health component, sampling could possibly be 160 carried out for additional toxic flora during the weeks without scheduled sampling. 161 For REPHYTOX, nearly 300 shellfish sampling stations could potentially be used, with 162 mussels at almost half of the stations, as well as oysters, scallops, clams, cockles or various 163 other shellfish. REPHYTOX sampling stations were in some cases in common with those of 164 REPHY. Alert thresholds, based on historical data, were defined for the phytoplankton toxic 165 species currently occurring on French coasts. Their detection by the REPHY network above 166 these thresholds triggered toxin monitoring in shellfish on a weekly basis. These alert 167 thresholds were: (i) for diarrheic shellfish toxin producing species: as soon as *Dinophysis* 168 was present, whatever the species, 10,000 cells per liter for Gonvaulax spinifera,

169 Lingulodinium polyedrum, Protoceratium reticulatum or Prorocentrum lima (when present in 170 the water column); (ii) for paralytic shellfish toxin producing species: 10,000 cells per liter in 171 areas usually affected by Alexandrium minutum, between 1,000 and 5,000 cells per liter in 172 areas usually affected by Alexandrium pacificum according to the zone, 10,000 cells per liter 173 for unidentified Alexandrium species; (iii) for amnesic shellfish toxin producing species: 174 300,000 cells per liter for the less toxic Pseudo-nitzschia species, 100,000 cells per liter for 175 the most toxic; the difference between these two groups of species was made from their 176 general form: broad or slender for the most toxic (including e.g. P. australis, P. fraudulenta, P. 177 multiseries, P. pungens), thin or sigmoid for the least toxic (including e.g. P. delicatissima, P. 178 *multistriata*); the notion of 'more or less toxic' is deduced from the history observations made 179 in France, and is subject to evolution. However, there were two cases where the monitoring 180 of toxic phytoplankton was not reliable enough to ensure the absence of toxins in shellfish. 181 Firstly, lipophilic toxins were sometimes observed in shellfish before the presence of 182 *Dinophysis* was detected; the search for these toxins was therefore systematically carried out 183 in the 'risk zones' and during 'risk periods' defined using the history of results over the last 184 three years. Secondly, offshore and deep-sea shellfish deposits were systematically 185 monitored for all three toxin categories every fortnight, one month before and during the 186 entire shellfish harvesting period. Mussels (Mytilus edulis, M. galloprovincialis) were 187 considered as sentinels for lipophilic toxins, since historical data showed that they were 188 always contaminated earlier than all other shellfish. If they were present in a production area, 189 the mussels were analysed first, and the other shellfish as soon as the mussels became 190 toxic. This was not the case for PST and AST episodes: all shellfish species were then 191 monitored simultaneously.

192 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 197 grouped into three different categories: (i) 'total flora' corresponded to the identification and 198 count of all phytoplankton taxa identified using light microscope; (ii) 'targeted flora' 199 corresponded to the identification and count of a targeted list of species, including those in 200 high concentrations and those which were known to be toxic or harmful; (iii) 'toxic flora' 201 corresponded to the identification and count of the few species that produced toxins that 202 could accumulate in shellfish. The method used was that of Utermöhl (1931). The 203 identification of phytoplankton was based on the WoRMS (World Reference Marine Register) 204 and had to be at the most precise taxonomic level (species or genus) if possible, and if not, 205 at a higher taxonomic level (family, class, etc.). The analysis by flow cytometry, according to 206 the Vaguer et al. (1996) method, provided functional groups for nano- and pico-207 phytoplankton. Chlorophyll a and pheopigments were essentially analyzed by 208 monochromatic spectrophotometry or fluorimetry (Aminot and Kérouel, 2004). The measured 209 nutrients were: ammonium (NH₄⁺), nitrate (NO₃⁻), nitrite (NO₂⁻), phosphate (PO₄³⁻), silicate 210 (SiOH₄). The ammonium concentration was determined by flow fluorimetry, and the 211 concentration of the other four nutrients by flow spectrophotometry (Aminot and Kérouel, 212 2007). In addition to phytoplankton observations, the measurement of physical and chemical 213 parameters (i.e. temperature, salinity, turbidity and dissolved oxygen) were generally 214 measured in situ using sensors, with a multi-parameter probe. 215 For REPHYTOX, detection and quantification of phycotoxins was carried out on the total 216 flesh from live shellfish according to the official European methods. These also describe 217 regulatory safety thresholds not to be exceeded for shellfish to be considered consumable. 218 Lipophilic toxins including DST have been measured by Liquid Chromatography coupled to 219 tandem Mass Spectrometry (LC-MS / MS) since early 2010 (Anonymous, 2015). They had 220 been previously detected by mouse bioassay according to the method of Yasumoto et al., 221 (1984 modified). These results were therefore qualitative until 2009, and expressed in terms 222 of 'favorable' or 'unfavorable'. The results were quantitative from 2010 and included

223 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

225 OA+DTX+PTX group (okadaic acid + dinophysistoxins + pectenotoxins); (ii) 160 µg of AZA 226 equivalent for AZA (azaspiracids); (iii) 3750 µg of YTX equivalent for YTX (yessotoxins). Each 227 of the constitutive toxins in these groups was corrected by a TEF (Toxic Equivalent Factor) to 228 account for its potential toxicity. The quantification of PST was carried out by mouse 229 bioassay, calibrated and validated to give a quantitative result (Anonymous, 2014). The 230 regulatory threshold was 800 µg STX (saxitoxin) equivalent per kg of shellfish flesh. AST 231 were measured by High Performance Liquid Chromatography with Ultra Violet detection 232 (HPLC/UV) (Anonymous, 2008). The regulatory threshold was 20,000 µg DA (domoic acid) 233 equivalent per kg of shellfish flesh. For PST and AST, the results recorded in the REPHYTOX 234 data did not include detailed results for each constitutive toxin.

- 235
- 236 **2.3** Data: storage, availability, communication

237 All REPHY and REPHYTOX results have since 1987 been recorded in the national 238 'Quadrige' database, in compliance with a guality process, the main characteristics of which 239 are: administration of the database by a dedicated data management team, mandatory 240 training and agreement for the use of Quadrige, entering the results according to a user 241 manual, compulsory checks and validation of the data entered, a quality process 242 implemented a posteriori on the data in order to provide, for each result, information on the 243 quality (good, doubtful or erroneous). Access to the raw data of Quadrige is restricted to 244 authorized users, but the validated data are public, associated with a DOI (Digital Object 245 Identifier), and accompanied by user manuals in English: REPHY (2017), REPHYTOX 246 (2017), with the respective DOI: doi.org/10.17882/47248, doi.org/10.17882/47251. The 247 datasets used for the presentation of the results below were all extracted from the Quadrige 248 database.

249

250 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

253 areas: 38 for the Channel, 54 for the Atlantic and 31 for the Mediterranean. Phytoplankton 254 taxa were always grouped in 'Taxonomic Units' (TU). The REPHY data were composed of 255 more than 500 different taxa over the entire period 1987-2018, including more than 200 256 species or groups of species, more than 200 genera or groups of genera, and about 40 257 families. To partially address the difficulty of processing such heterogeneous data, 258 Hernández-Fariñas et al. (2014) proposed using taxa clusters and described them as 259 Taxonomic Units, which were most often groups of genera and / or groups of species. The list 260 was thus reduced to less than 250 TU.

261 Two characteristics of phytoplankton data for the last 20 years (1999-2018) are presented 262 here: dominance and abundance. For the determination of 'dominant' TU, only 'total flora' 263 data were taken into account for this calculation. The dominance measure used here was 264 derived from the Sanders index (1960). This is determined by classifying the species from 1 265 to 10 by abundance in each sample. A rank of 1 receives a value of ten points, a rank of 2 266 nine points, etc. A rank of 10 receives one point. The points are summed on all samples: the 267 higher the sum of points, the more dominant is the species. This approach consisted on the 268 one hand of taking abundance into account by using relative numerical abundance of 269 species in samples instead of points, and on the other hand considering all species and not 270 only the ten most abundant ones. The summary of results for TU abundance was based on 271 the 90th percentile, encompassing the spread of data omitting highly skewed values (Devlin 272 et al., 2014), computed with TU occurrence abundance during the considered period and 273 marine area. These values were represented on a map as disks with a diameter proportional 274 to their abundance class on a log 10 scale. The number of TU occurrences was taken into 275 account through the transparency of the disk color varying linearly from transparent (i.e. 276 1 occurrence) to opaque (i.e. 240 occurrences, that is, identified each month during 20 277 years). Six TU were chosen according to their toxicity or harmfulness. Three of these TU 278 included species that produced toxins that were dangerous to shellfish consumers 279 (Dinophysis + Phalacroma, Alexandrium and Pseudo-nitzschia). The other three TU 280 contained species that already led to mortalities of marine fauna in France: (i) Karenia

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281 mikimotoi, a producer of hemolytic ichthyotoxins; (ii) *Phaeocystis*, via mechanical action with 282 production of foams; (iii) Lepidodinium chlorophorum which, by its capacity to develop rapidly 283 in abundant quantities, has regularly led to anoxia in the marine environment .The results on 284 toxins were summarized, on the one hand temporally, and on the other hand spatially. For 285 the temporal dimension, a toxicity episode of a given toxin (i.e. DST, PST or AST) was 286 defined by its occurrence in shellfish above the European regulatory threshold. For each 287 given year and toxin, a marine area was considered affected by the given toxin if at least one 288 toxicity episode was recorded. Then, to compare the number of affected areas by province, 289 year and toxin, sums were divided by the number of marine areas of the corresponding 290 province and plotted as stacked bar charts. For the spatial dimension, three maps were 291 produced (one per toxin) showing for each marine area the number of years affected by at 292 least one toxic episode in the year. In order to achieve more relevant comparison between 293 the respective impacts of the three categories of toxins, the focus for these maps was on the 294 period 2000-2018 (the PST and AST being less or not present before 2000). Data 295 processing, tables and maps were made with R (2018) and QGIS (2018).

296 3 Results

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

298 3.1 Phytoplankton

299 Table 1 details the most dominant TU for the period 1999-2018. It shows that five TU can be 300 considered as the most emblematic of the French coastline as a whole: Chaetoceros, 301 Skeletonema, Cryptophyceae, Leptocylindrus, Pseudo-nitzschia. These always belong to the 302 group of the ten most dominant TU, whatever the geographical region. Among the target TU, 303 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 304 305 second most dominant TU on the French coast of the Channel. Lepidodinium, which is 306 another harmful TU of interest, has also never been observed on the Mediterranean French 307 coasts. Unlike *Phaeocystis*, it is more dominant on the Atlantic coasts than in the Channel. 308 The three other toxic or harmful TU of interest, i.e. Dinophysis + Phalacroma, Alexandrium

309 and *Karenia*, are towards the bottom of the ranking, respectively, 47th, 49th and 61st. Over the 310 same period of 20 years, Fig. 2 provides a summary of the geographical distribution of some 311 of these TU, chosen because they include toxic or harmful species. The geographical 312 distribution of *Pseudo-nitzschia* shows that it was regularly present along the entire coastline 313 at high concentrations. *Dinophysis* + *Phalacroma*, *Alexandrium* and *Karenia* have been 314 observed in all marine provinces. Dinophysis + Phalacroma occurred mostly in the Bay of 315 Seine, in western and southern Brittany, and in the lagoons of the western Mediterranean or 316 Corsica. Alexandrium appeared more frequently in Brittany and in the lagoons of the western 317 Mediterranean. Karenia was observed everywhere, but its regular presence was only 318 observed in certain areas, especially in western and southern Brittany, in low concentration. 319 *Phaeocystis* was observed mainly in the Channel and in southern Brittany, with the highest 320 concentrations along the northern parts of the French coasts. Lepidodinium was very little 321 present in the Channel and was absent in the Mediterranean. It was mainly present in 322 southern Brittany and around the Loire estuary.

323 3.2 Toxins

324 Fig. 3 shows that on the Atlantic coast, the percentage of affected areas was almost always 325 greater than in the other provinces. The appearance of AST episodes was observed on this 326 coast since the mid-2000s. This pattern is also found in the Channel, on a smaller scale. DST 327 episodes do not seem to have increased or decreased over these 32 years, regardless of the 328 province. PST episodes occurred on all three provinces, but the percentage of affected areas 329 has not increased significantly since their appearance respectively in 1988 in the Channel, 330 and 1998 in the Mediterranean. Fig. 4 shows the geographical distribution and the 331 recurrence of episodes of toxicity during the last 19 years, by toxin, and by area. It shows 332 that many areas were regularly affected by DST episodes, especially in Normandy, in 333 western and southern Brittany and in several regions of the Atlantic coast, on the 334 Mediterranean coast, and in lagoons of the western Mediterranean and Corsica. Conversely, 335 whole regions were spared from DST episodes, especially along the northern coast of 336 France, and along the Channel. With regard to PST episodes, a few areas were affected

337 over the period, sometimes sporadically. The two most frequently affected areas were: Brest 338 harbor in western Brittany, and Thau lagoon in the Mediterranean. The AST episodes 339 showed a pattern that was different from that of DST and PST, with a large number of 340 affected areas but fewer than for DST. The most frequently affected areas over the period 341 were: the Bay of Seine in Normandy, and the western and southern coasts of Brittany. 342 Table 2 summarizes the information on the relative weight of the shellfish affected by the 343 episodes of toxicity. In some cases, the different species of a genus are grouped, either 344 because some species hybridise with each other or because they are difficult to identify. This 345 is the case of: (i) Mytilus, the species occurring in France being M. edulis (in Channel and 346 Atlantic), *M. galloprovincialis* (rather Mediterranean), or a hybrid of the two; (ii) *Donax*, which 347 includes D. trunculus and D. vittatus; (iii) Ruditapes, which includes R. decussatus and R. 348 philippinarum or their hybrids; (iv) Mimachlamys which includes the species M. varia. Table 2 349 shows that: DST episodes regularly affected a wide variety of shellfish especially in the 350 Atlantic, PST episodes affected only a few species of shellfish, while AST episodes mainly 351 affected scallops, especially in the Channel. This inventory should be qualified by the fact 352 that the small number of areas affected by PST reduced the range of shellfish species 353 impacted.

354 4 Discussion

355 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.

358 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) 365 Chaetoceros regularly exceeded 10 million cells per liter, particularly in the Channel; (ii) 366 Skeletonema often exceeded 1 million cells per liter, and often 10 million in the 367 Mediterranean, and even reached 550 million in northwestern Brittany in 2000; (iii) 368 Cryptophyceae quite often exceeded 1 million; (iv) Leptocylindrus often exceeded 1 million 369 cells per liter, in all provinces. Blooms greater than 1 million for these four TU were generally 370 observed from March to October in the Channel and Atlantic, and in all seasons in the 371 Mediterranean. For the TU containing toxic or harmful species, and mapped in Fig. 2, they 372 systematically include all species belonging to the TU, regardless of their harmful potential. 373 However, the geographical distribution of these TU provides in most cases interesting 374 information in terms of risk.

375 For Pseudo-nitzschia, which was regularly present everywhere (Fig. 2), the pattern was 376 similar from one year to the next, with annual maxima per marine area often greater than 1 377 million cells per liter, sometimes 10 million, in all provinces. Blooms over 1 million were 378 observed from March to November in the Channel and Atlantic, all year round in the 379 Mediterranean. The national record was reached in 2010 in the western Mediterranean with 380 60 million cells per liter. Several Pseudo-nitzschia species occurred in France. The species 381 P. australis and P. pseudodelicatissima have been formally identified as the cause of AST 382 episodes (Amzil et al., 2001, Nézan et al., 2006). It should also be noted that the following 383 toxigenic species were present: P. delicatissima, P. fraudulenta, P. multiseries, P. multistriata, 384 P. pungens (Lassus et al., 2016). Given the difficulty of identifying species of this genus 385 under the light microscope, the identifications in the REPHY data were mostly related to 386 species groups called "complex": for example the *delicatissima / pseudodelicatissima* 387 complex, the seriata complex, etc. It was therefore not possible to quantify the percentage of 388 the contribution of toxic species in blooms compared to that of non-toxic species. However, 389 some groups contain more toxic species, and the alert thresholds defined in the REPHY for 390 triggering AST analysis takes this into account. The REPHY and REPHYTOX data showed 391 that shellfish only became toxic after the development of at least several 100,000 cells per 392 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.

400 Table 1 and Fig. 2 consider the two *Dinophysis* + *Phalacroma* genera together in the TU of 401 the same name. Data from the three provinces for this TU were almost exclusively 402 Dinophysis species. Otherwise, some Phalacroma species may also be toxic (Lassus et al., 403 2016). The geographical distribution of these two genera together provided therefore relevant 404 information in terms of risk. If this TU was present in all provinces, the concentrations were 405 never high, or it was not present in all seasons, as evidenced by its weak dominance rank. 406 especially in the Channel and Atlantic, respectively 57th and 58th (Table 1). The maximum 407 annual concentrations were generally < 10,000 cells per liter, and were always < 100,000 408 cells per liter with the exception of Normandy, for which higher concentrations were recorded, 409 e.g. 803,000 cells per liter on the coast of Calvados (south of Seine Bay) in 2006. If 410 Dinophysis could be present in all seasons, it was generally observed from March-April in 411 western and southern Brittany, and from July-August in Normandy. While it was rarely 412 observed in winter on the Channel and Atlantic coasts, *Dinophysis* was present year round in 413 the Mediterranean. In France, it was mainly Dinophysis acuminata and D. sacculus species 414 that were responsible for DST episodes. But more episodic or more localized presence of the 415 following potentially toxigenic species, could be observed: D. acuta, D. caudata, D. fortii, D. 416 norvegica, D. tripos. Shellfish could become toxic even when Dinophysis was observed at 417 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 421 annual maxima in the areas concerned often exceeded 1 million cells per liter, sometimes 10 422 million (always between June and August). The very regular presence of Alexandrium in 423 Thau lagoon, all year round, but especially in autumn and winter, made it more dominant in 424 the Mediterranean (29th) than on the other provinces. In France, the two toxic species that led 425 to PST episodes were: A. minutum in the Channel and Atlantic (Lassus et al., 2016); 426 A. pacificum in Thau lagoon (Masselin et al., 2001; John et al., 2014). REPHY and 427 REPHYTOX data showed that shellfish became toxic only after significant development of a 428 toxic species of Alexandrium (several thousand or tens of thousands of cells per liter, 429 depending on regions and species), which explains why Alexandrium was an excellent 430 indicator of future PST contamination. The areas most frequently affected by blooms greater 431 than 100,000 cells per liter were located in northern and western Brittany, as well as in Thau 432 lagoon. The maximum concentration recorded since 1999 was 41 million cells per liter in 433 Brest harbor in 2012, and significant blooms have been recorded in this area since (Chapelle 434 et al., 2015). Guallar et al. (2017) showed that temperature was a key factor for bloom 435 initiation, and river flow for the maximum abundance. However, this second factor was local, 436 favoring growth through the supply of nutrients and limiting abundance by dilution. They also 437 showed that Alexandrium has dynamic characteristics of an invasive species: an introduction 438 period with low abundance, then a period with high abundance blooms and, finally, a period 439 of presence but at lower abundance (because controlled by parasites or predators or 440 competition). In the Mediterranean, the most prolific blooms of A. pacificum occurred in Thau 441 lagoon. The fact that the strain of this species in this lagoon was related to a Japanese strain 442 (Lilly et al., 2002) has supported the hypothesis of an accidental introduction of A. pacificum. 443 The cysts transported in the deballasting waters of Asian ships possibly contaminated the 444 shellfish of the Spanish Mediterranean coast, leading to the introduction into Thau lagoon 445 and the subsequent distribution of contaminated shellfish between France and Spain in the 446 years 1980-1990. The Alexandrium blooms at Thau required special hydroclimatic 447 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.

450 While it does not appear to be a dominant TU (Table 1), Karenia, essentially represented in 451 the data by K. mikimotoi, remained associated with significant harmful effects in France, by 452 its hemolytic toxins acting directly on the marine fauna and flora, even if the events in 453 guestion were not recent. The regular presence of *Karenia* was only found in certain areas, 454 especially in western and southern Brittany, in low concentration. Over the period 1999-2018, 455 the maximum observed was 136 million cells per liter for K. mikimotoi, in Brest harbor in 456 2002. In the years 1970-1980, K. mikimotoi was associated with harmful impacts on scallops 457 (Pecten maximus): mortalities, growth inhibition, etc. in western Brittany (Lassus et al., 458 2016). In June-July 1995, it caused massive mortalities of marine animals along the French 459 Atlantic coast; this episode reached a considerable scale, never repeated since, by its 460 geographical extension from western Brittany to south of the Loire river; the biological and 461 economic impacts of these blooms were wide-ranging. It is worth remembering that the 462 REPHY results were for near-shore waters, and could not be representative of phenomena 463 that would have appeared offshore. This point is crucial for K. mikimotoi, whose near 464 monospecific blooms were observed by satellite each year in July at the western entrance to 465 the Channel (Ménesguen et al., in revision), while Fig. 2 shows that the concentrations were 466 not high in inshore coastal zones. The presence of K. mikimotoi offshore was particularly 467 noted in the frontal zones and in the stratified waters where it could have access to high 468 levels of ammonium (its preferred nitrogen nutrient form) resulting from the bacterial 469 remineralization of the previous diatom blooms having sedimented at the bottom (Le Corre et 470 al., 1993, Ménesguen et al., in revision). Worth noting is the presence in France, but rarely 471 and always at low or very low concentration, of another species of *Karenia*, potentially 472 producing brevetoxins: K. papilionacea. Because of the low concentrations observed, no 473 harmful effect has ever been observed in France.

474 Another toxic species is worth mentioning: *Ostreopsis*, even if it does not appear in the first
475 40 dominant TU of Table 1. Present in the REPHY data since the 1990s, *Ostreopsis* has

476 been particularly closely monitored since 2006, following respiratory disorders in bathers in 477 the Marseille region (east Mediterranean). Only one species could be identified on a 478 morphological and molecular basis, O. cf. ovata (Penna et al., 2005), producer of palytoxins 479 and ovatoxins, which release into the air in the form of aerosols can lead to irritation of the 480 skin, eyes and respiratory system. Ostreopsis was observed almost every year in the French 481 Mediterranean, particularly in more eastern areas not monitored by REPHY, where it 482 regularly formed major blooms in summer (Amzil et al., 2012; Lemée et al, 2012; Brissard et 483 al., 2014).

484 Among the toxic species of recent appearance in France are: Karlodinium, Vulcanodinium 485 rugosum and Azadinium. It should be noted that not all of them are currently identified as 486 such in the database, but most often under a higher level taxon (family, order, class, etc.), at 487 least until a certain period of time. For example, Karlodinium, of which several species are 488 known to be toxic to fish, has been present in the data only since 2008. However, fish 489 mortalities observed in 1994 in Diana lagoon (Corsica), following a proliferation of 490 Gyrodinium corsicum, known since as Karlodinium corsicum (Lassus et al., 2016), proved 491 that this genus was really present in French waters. *Vulcanodinium rugosum*, described by 492 Nézan and Chomérat (2011), was present in the data from 2013 and identified as the source 493 of pinnatoxin production in shellfish of Ingril lagoon on the Mediterranean west coast (Hess et 494 al., 2013). Its presence has been known since 1996, but it was only in 2009 with its massive 495 proliferation at Ingril that it was possible to describe it. Azadinium, recognized as a producer 496 of azaspiracids (Tillmann et al., 2009) was present in the REPHY data from 2013 in the 497 Atlantic. Azaspiracids were detected in France in small guantities as early as 2002 (Lassus et 498 al., 2016), then in 2008 (Amzil et al., 2008): so, Azadinium was probably already present in 499 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 500

501 specific to this province, essentially along the northern part of the French coast, as shown in 502 Fig. 2. It was present every year in this region, of which the coastal waters are among the 503 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 1 million, 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.

508 At the 12th rank of dominance in the Atlantic (Table 1), *Lepidodinium* was mainly present in 509 southern Brittany and around the Loire river estuary (Fig. 2). The annual maxima of 510 Lepidodinium often exceeded 10 million cells per liter, the record since 1999 being 100 511 million in northern Brittany in 2001. Blooms greater than 1 million cells per liter could be 512 observed from March to November. The TU was entirely represented by the species 513 L. chlorophorum, which colored the waters a very intense green. Although non-toxic, its 514 proliferation regularly led however to significant anoxia, possibly resulting in mortalities of 515 marine animals. The links between L. chlorophorum blooms and anoxia were described in 516 Sournia et al. (1992), and could be found in REPHY data for more recent data: e.g. in Vilaine 517 Bay (southern Brittany) in July 2018, 30 million cells per liter of L. chlorophorum were 518 associated with three dissolved oxygen values below 2.9 mg/L, hypoxia threshold defined by 519 Diaz and Rosenberg (2008). Green waters recorded on the Atlantic coast since 1982 could 520 also be attributed retrospectively to this same species (Sournia et al., 1992). Having a 521 typically coastal distribution, its proliferation seemed to correlate with the river Loire inputs. 522 In addition to the results presented here, REPHY phytoplankton data sets (REPHY, 2017), 523 have also been used to answer scientific questions, and have provided valuable information 524 on phytoplankton biodiversity, spatial distribution and temporal variations of the various taxa 525 along the French coastline (Beliaeff et al., 2001; Gailhard et al., 2002; 2003). More recently, 526 David et al. (2012) analyzed the variability of phytoplankton groups in relation to climatic and 527 environmental indices in the Bay of Biscay. Hernández-Fariñas et al. (2014) have shown 528 modifications in species diversity over the last twenty years in the eastern Channel, in 529 relation to hydroclimatic changes. Studies have also examined the conditions of the 530 appearance of toxic species, e.g. Husson et al. (2016) for Pseudo-nitzschia, Chapelle et al. 531 (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.

535

536 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.

540

541 4.2.1 History of toxic episodes in France

542 Shellfish farming is a historical activity in France for centuries. The two main shellfish 543 exploited are oysters and mussels, with currently about 130,000 tons of oysters and about 544 65,000 tons of mussels produced each year, for a total production of about 200,000 tons all 545 shellfish combined (http://coquillages.com /statistics/). These tonnages have been similar 546 from year to year for decades (Buestel et al., 2009). It should be noted that all French areas 547 exploited by shellfish farmers or fishermen, in production areas, natural populations, and 548 professional fishing zones, have always been monitored, except in two cases. The first case 549 concerned scallop (Pecten maximus) fishing areas until 2004 for all categories of toxins; this 550 has little impact on the interpretation of the evolution of the toxic episodes for DST and PST, 551 the scallops being mainly concerned by AST. The second case concerned oysters 552 (Crassostrea gigas) until 2001, which were not monitored as first-line for DST; given that 553 DST data indicate that mussels have always been toxic prior to oysters over the 2001-2018 554 period, it is likely that the lack of oyster DST data prior to 2001 has a relatively small impact 555 on overall interpretation DST data.

556 DST episodes have been regularly observed in all provinces since 1987 (Fig. 3). In fact, the 557 first toxic episodes clearly associated with diarrheic toxins date back to 1983, when about 558 3,000 cases of diarrheic intoxication were reported in western France. But it is very likely that 559 diarrheic toxins had contaminated shellfish long before that date: intoxications observed 560 previously could have been confused with bacterial or viral poisoning (Caruana and Amzil, 561 2018). Lipophilic toxins incriminated in shellfish contamination were always OA (most often) 562 or DTX (Amzil and Mathias, 2006; Amzil et al., 2007; REPHYTOX, 2017). The REPHY and 563 REPHYTOX data showed that these toxins were always present in association with the 564 phytoplankton *Dinophysis*. Two other groups of lipophilic toxins were sometimes present in 565 France, but always at very low concentration: AZA and YTX. Their presence could not be 566 formally associated with their potential producers, although these were sometimes observed 567 in water samples, respectively Azadinium for AZA, Lingulodinium polyedrum and 568 Protoceratium reticulatum for YTX.

569 The first observation of PST dates from 1988 in northwestern Brittany, following the detection 570 of significant concentrations of *Alexandrium minutum*. The Mediterranean province was 571 affected ten years later, in 1998, in Thau lagoon. The presence of PST in France before 1988 572 is highly unlikely, given the following considerations: (i) the first observations of PST in 573 Europe were made only in 1987 (Norway, Sweden, Denmark, Spain, Portugal) (information 574 from: http://haedat.iode.org/index.php), (ii) in 1988, the monitoring was in place in France for 575 4 years, (iii) from 1988 to 1990, PST were observed only in the same two marine areas. The 576 toxin profile of A. minutum was composed mainly of gonyautoxins-2,-3 (carbamate toxins) 577 (Lassus et al., 2004) and toxins-C1,-2 (N-sulfocarbamoyle toxins), while that of A. pacificum 578 was composed mainly of gonyautoxins-1, -4, -5 and toxins C-2, C-4 (Masselin et al., 2001). 579 In the majority of cases, the presence of PST above the regulatory threshold was associated 580 with significant developments of Alexandrium. The case of the Arcachon Bay episode in 581 2002-2003 remains the only one unsolved, since the presence of Alexandrium was not 582 observed in the phytoplankton flora: no alternative explanation could be found for the 583 presence of PST above the regulatory threshold, even if it was very low (864 µg STX 584 equivalent per kg of shellfish flesh). With this exception, the Alexandrium / PST episode 585 relationship cannot be called into question in the light of available 32-year data, and confirms 586 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; 587 588 Nezan et al., 2006). These toxins have been monitored along the entire coast only since 589 1999. It is therefore conceivable that occurrences of AST have not been detected before this 590 date, but this assumption is unlikely. Indeed, the first observations of AST in Europe were 591 made in 1992 (Sweden), then in 1995 (Spain, Portugal) and 1998 (UK) (information from: 592 http://haedat.iode.org/index.php), showing slow progression of AST toxin occurrences across 593 Europe. In addition, a study conducted by LeDoux et al. (1996) on several areas of the 594 French coast showed that these toxins were then absent from those areas, at least in the 595 mussels. Finally, the system of control of food poisoning by the French authorities would 596 have highlighted a problem related to the contamination of shellfish, which was not the case. 597 The increase in AST episodes recorded since 2004 in the Channel and the Atlantic (Fig. 3) 598 clearly corresponds to the beginning of the obligation to monitor scallops (*Pecten maximus*) 599 in the European context, since these were previously excluded from the toxin monitoring 600 system in France. In the different AST episodes, the toxin profile was mainly composed of 601 domoic acid, and its epidomic acid analogue in small amounts, both produced by the toxic 602 species of *Pseudo-nitzschia*. REPHY and REPHYTOX data showed that AST episodes were 603 always associated with the genus *Pseudo-nitzschia* in coastal areas for which phytoplankton 604 sampling could be performed at a depth close to that of toxins. Based on the REPHY-605 REPHYTOX available data over 32 years, the *Pseudo-nitzschia* / AST episodes relationship 606 confirms the relevance of the AST surveillance strategy based on a phytoplankton indicator. 607 In offshore areas (mainly scallop fishing areas), the association between phytoplankton and 608 AST could not be evidenced from REPHY-REPHYTOX data alone, phytoplankton sampling 609 not being carried out on deep scallops beds. But various in situ studies have shown the 610 correspondence between the accumulation of AST in shellfish and the proliferation of toxic 611 Pseudo-nitzschia species (Husson et al., 2016; Thorel et al., 2017).

612

613 4.2.2 Affected areas and shellfish

614 The geographical distribution of DST episodes (Fig. 4) highlights areas that were always 615 spared (e.g. northern France and northern Brittany). This could partly be explained by a 616 lower frequency of *Dinophysis* occurrences in these areas (REPHY, 2017). In northern 617 Brittany in particular, the hydrodynamics would not favor *Dinophysis* accumulation. In 618 regularly affected areas, the periods of toxicity were clearly different according to the region. 619 In the Channel, toxic episodes were generally observed between July and October. In the 620 Atlantic, they were: (i) from May, or even March-April, in western and southern Brittany, until 621 August, sometimes until October-November; (ii) generally from May to July-August between 622 the Loire and Gironde rivers; (iii) between April and August in the Arcachon Bay. In the 623 Mediterranean: (i) in the open sea zones, toxic episodes were generally spread between 624 March and October; (ii) in the lagoons, they could be observed all year long. The shellfish 625 contaminated during the presence of DST were very varied (Table 2), especially in the 626 Atlantic because the diversity of exploited shellfish was much greater than on the other 627 provinces. Mussels were systematically contaminated before other shellfish and often at 628 higher concentrations. The periods and duration of shellfish contamination-decontamination 629 of offshore beds were most often different from those of shellfish close to the coast. The 630 results prior to 2010 being qualitative (bioassay), the different toxic episodes could not be 631 compared in terms of toxin concentrations for this period. The highest toxin concentration 632 over the period 2010-2018 (expressed in µg of OA equivalent per kg of shellfish flesh) was 633 recorded in the Arcachon Bay in April 2012: 37,296 µg/kg for Mytilus sp.; in this zone, at the 634 same time, the following shellfish also reached their record, with 11,755 µg/kg for 635 Cerastoderma edule and 2,278 µg/kg for Ruditapes philippinarum. The maxima observed for 636 other species were: 4,792 µg/kg for *Donax trunculus* in western Brittany in June 2013, 637 2,621 µg/kg for Polititapes rhomboides in southern Brittany in June 2010, 2,305 µg/kg for 638 Pecten maximus off the eastern Channel coast in September 2014, 1,067 µg/kg for 639 Aequipecten opercularis in Jersey-Guernesey (Channel) in July 2018, 1,041 µg/kg for 640 Crassostrea gigas in Salses Leucate lagoon in December 2012. For Spisula solida, 641 Glycymeris alycymeris, 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.

645 Only two areas were affected for at least 5 years by PST episodes over the period 2000-646 2018 (Fig. 4), and were still so in recent years. The first was Brest harbor for which the first 647 PST episodes were recent (2012), and recurred very regularly, the last having taken place in 648 2017. The second was Thau lagoon in the Mediterranean, for which the PST episodes were 649 common between 1998 and 2007, and occurred again in 2015, 2016 and 2017. The three 650 areas of the north coast of Brittany were mostly affected in the 1990s, with some sporadic 651 episodes in the 2000s in one or other of these areas. For other areas (Arcachon Bay and 652 Mediterranean areas except Thau lagoon), there were only very isolated episodes, dating 653 back at least 10 years. The periods of PST episodes were very different between the Brittany 654 areas and those of the Mediterranean. In Brittany, PST episodes have always been observed 655 between June and August-September. In Thau lagoon, the PST episodes were 656 systematically observed between September and December. The shellfish contaminated 657 during the presence of PST were always among the following (Table 2): mussels, oysters, 658 cockles, clams. This was partly explained by the fact that the areas concerned only contained 659 these shellfish. But the wide range of data acquired with the systematic search for PST 660 during fishing periods on various offshore shellfish of the Channel and the Atlantic (Pecten 661 maximus, Aequipecten opercularis, Mimachlamys varia, Callista chione, Glycymeris 662 glycymeris, Polititapes rhomboides, Spisula, Venus verrucosa, Ostrea edulis), attest that 663 these shellfish have never been contaminated by PST, at least during fishing periods. The 664 highest concentration of PST (expressed in µg STX equivalent per kg of shellfish flesh) over 665 the period 1987-2018 was recorded in Brest harbor in July 2012: 11,664 µg/kg for *Mytilus* sp. 666 The maxima observed for the other shellfish species were: 7,360 µg/kg for *Crassostrea gigas* 667 in northern Brittany in August 2001, 5,740 µg/kg for Ruditapes decussatus in Thau lagoon in 668 November 2001, 3,300 µg/kg for *Cerastoderma edule* in northern Brittany in June 1998.

24

669 The areas and shellfish most affected by AST episodes over the period 2000-2018 (Fig. 4 670 and Table 2), and which were still so in recent years, were: (i) in Normandy, exclusively 671 scallops (*Pecten maximus*) off the Bay of Seine; (ii) in western and southern Brittany, mostly 672 Pecten maximus, but also Donax or Polititapes rhomboides, and sometimes Mytilus edulis, 673 Crassostrea gigas, Callista chione, Aequipecten opercularis. On the Atlantic coast, between 674 the rivers Loire and Gironde, the year 2010 was an atypical year: while AST were observed 675 for the first time in this region, the variety of contaminated shellfish (mussels, oysters, clams, 676 scallops, etc.) has never been found in later episodes. The hypothesis of a link between 677 these episodes and the storm Xynthia which passed over much of France at the end of 678 February 2010 is very likely. The conjunction of the storm with high tidal coefficients did 679 indeed lead to the flooding of many coastal areas to the south of the Loire estuary (Bertin et 680 al., 2012). The marine waters, which were then loaded with nutrients, were probably the 681 trigger of Pseudo-nitzschia blooms (Nézan et al., 2010; Husson et al, 2016), with 682 concentrations never observed before or since 2010 in this region. On the Mediterranean 683 coast, AST episodes affected Mytilus galloprovincialis or Donax from several areas of the 684 west coast in the early 2000s; since 2006, no more AST episodes have been observed. If 685 whole regions were spared the presence of AST, this is not explained by lower frequencies or 686 concentrations of *Pseudo-nitzschia* in these areas; in fact the year-by-year maps of 687 developments of this taxon show significant blooms every year along the entire French coast 688 (Fig. 2). The most plausible explanation remains that these blooms were often composed of 689 several *Pseudo-nitzschia* species, whose toxic potential might be very different. Almost all 690 AST episodes were observed between March and May including in the Mediterranean, over 691 the entire period 2000-2018, and for all shellfish apart from scallops. This information 692 corroborates the observations relating to *Pseudo-nitzschia*, of which proliferations in high 693 concentration began most often in March-April. Conversely, scallops (Pecten maximus) could 694 be contaminated all year round in the Channel (Amzil et al., 2009) or the Atlantic, with the 695 fishing periods extending most often from October to March. The highest concentration of 696 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.

702

703 **5 Conclusions**

704 REPHY phytoplankton data contains gems of knowledge regarding the temporal and spatial 705 distribution of species in terms of presence, abundance and biodiversity. The elementary 706 data analysis used here has enabled us to pick only the low hanging fruits. Among them, 707 dominant Taxonomic Units of the French coast during the last 20 years were *Chaetoceros*, 708 Skeletonema, Cryptophyceae, Leptocylindrus and Pseudo-nitzschia. Occurrences and 709 frequencies of occurrence of 6 toxic and harmful TU showed different spatial patterns. 710 suggesting a mosaic of phytoplankton ecological niches and of coastal ecosystems. The 711 long-term information gathered over the years enabled the investigation of abundance time 712 series, unusual events and conditions of appearance of these species. The patterns of 713 change of shellfish toxicity episodes over 32 years appeared as a growing trend on the 714 Atlantic coast in the overall number of toxic episodes since 1987; the appearance of AST 715 episodes and the beginning of the obligation to monitor scallops, especially since 2005, 716 explains this upward trend. It should be noted that PST and AST were very probably absent 717 from the French coasts, respectively before 1988 and 2000. In addition, even by roughly 718 estimating the number of occurrences of DST before the 1980s, a projection over 40 or 50 719 years would undeniably show that the presence of toxins in shellfish became, in the years 720 1980-1990, a priority public health concern and a major issue for shellfish farmers and 721 fishermen in France. It was to be feared that other toxins could also appear: For this reason, 722 in parallel with the monitoring carried out under REPHY-REPHYTOX, a shellfish watch is 723 also undertaken by Ifremer for toxic micro-algae and toxins known in other countries, but not 724 vet 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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		1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Channel	DST	5.26	15.79	15.79	10.53	0.00	5.26	5.26	10.53	7.89	0.00	2.63	5.26	5.26	5.26	2.63	2.63	7.89	5.26	13.16	2.63	2.63	5.26	5.26	5.26	7.89	2.63	7.89	10.53	5.26	10.53	7.89	15.79
	PST	0.00	2.63	2.63	5.26	0.00	0.00	2.63	2.63	2.63	2.63	2.63	2.63	7.89	2.63	2.63	0.00	2.63	0.00	0.00	0.00	0.00	0.00	0.00	5.26	0.00	2.63	2.63	0.00	2.63	0.00	0.00	0.00
	AST	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.53	2.63	0.00	2.63	2.63	2.63	0.00	10.53	15.79	7.89	2.63	0.00	2.63	2.63	0.00
Atlantic	DST	29.63	37.04	22.22	42.59	31.48	42.59	16.67	42.59	11.11	29.63	22.22	20.37	29.63	35.19	40.74	38.89	16.67	35.19	24.07	50.00	44.44	33.33	29.63	42.59	38.89	31.48	31.48	27.78	33.33	44.44	24.07	42.59
	PST	0.00	0.00	0.00	0.00	0.00	0.00	5.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.85	1.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.85	1.85	1.85	1.85	0.00	1.85	0.00
	AST	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.70	0.00	0.00	0.00	1.85	11.11	9.26	14.81	12.96	7.41	37.04	18.52	16.67	11.11	9.26	14.81	1.85	5.56	1.85
Mediterranean	DST	16.13	6.45	29.03	25.81	22.58	16.13	25.81	19.35	12.90	3.23	3.23	12.90	16.13	9.68	6.45	16.13	6.45	6.45	6.45	9.68	6.45	19.35	12.90	3.23	3.23	6.45	19.35	12.90	9.68	9.68	19.35	12.90
	PST	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.23	3.23	3.23	3.23	0.00	3.23	3.23	0.00	0.00	6.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.23	3.23	3.23	0.00
	AST	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.68	0.00	3.23	0.00	6.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00













	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				

(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

	By geographic regior						
	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			



Toxin	DST (sir	ice 1987)		PST (sin	ce 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										