

Are there Invasive Planktonic Microbes?

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Submitted: 04 Nov 2019; Accepted: 11 Nov 2019; Published: 25 Nov 2019

Abstract

The translocation of species by human activities is a problem that increases with the globalization. However, the examples of non-indigenous or exotic planktonic microbes can be questioned as they predominantly have cosmopolitan distributions and natural mechanisms for wide dispersion. In reality, the categorization of any species as non-indigenous requires solving two difficult issues: knowledge of where the 'natural' population is, and demonstration of a substantial geographic discontinuity between the supposed source and the introduced populations. With regard to planktonic microorganisms, a non-indigenous taxon could have been previously unnoticed during routine microscopical analyses due to: A) difficult identification at the species level in routine observations such as for the diatoms (*Pseudo-nitzschia*, *Skeletonema*, *Thalassiosira*, *Pleurosigma*), unarmoured dinoflagellates (*Karenia*, *Karodinium*) and *Raphidophytes*, and B) species with strong interannual fluctuations of abundance, only detected during bloom periods when they are misinterpreted as newcomers (i.e., *Coscinodiscus wailesii* or *Trieres chinensis*, junior synonyms of *C. cylindricus* and *T. regia*, respectively, or *Gymnodinium catenatum*). Rather than attempting to add to the lists of non-indigenous species with planktonic microbes, the monitoring surveys should also pay attention in the less common species with important fluctuations of abundance, independent of tentative labels as exotic or indigenous, because they are potentially useful as bio-indicators of environmental changes.

Keywords: Ballast Water Management, Biological Invasions, Climatic Bio-indicators, Exotic Protist, Non-indigenous Dinoflagellate, Alien diatom, Introduced phytoplankton

Introduction

Non-indigenous (= alien, exotic, non-native, allochthonous) is a term used for species artificially introduced outside of their natural range, and by extension outside of their natural dispersal potential. This implies a geographical discontinuity between the native and the non-indigenous populations. Often, the non-indigenous species, free of their usual predators and/or parasites, are able to colonize a new habitat. There are examples such as the Zebra mussel in continental waters, the macroalgae *Caulerpa taxifolia* in the Mediterranean Sea or the lionfish in the Caribbean Sea. Despite the huge diversity of planktonic microbes, only a few tens of species have been categorized as non-indigenous and even these examples have been questioned [1-4].

There are important differences in the biogeography of micro- and macroscopic organisms. The detection of a microbe is not accessible to everyone, as we need tools such as a microscope, while macroscopic species have a high number of potential observers. Microbes have often relatively few distinctive morphological characters and species identification often requires considerable expertise. Consequently, the data set of microbial geographical distribution is poor and largely biased when compared to the macroscopic species. The abundance of microbes is often temporally variable, characterised by important

fluctuations, and numerous species are only detected when they bloom. In the case of the marine plankton, we should recall that the term 'plankton' refers to a wandering life that facilitates the dispersion in an environment with few physical barriers. An example of ocean dispersion is the case of the friendly floaters washed into the Central Pacific that landed in the British coasts. This combination of features is a key to understand the relative low number of non-indigenous plankton microbes.

This manuscript reviews the requirements of the planktonic microbes to be considered non-indigenous, and the trends on the biogeography -cosmopolitan versus endemic distributions-. We comment the examples of some phytoplankton species considered as non-indigenous in the literature: *Alexandrium minutum*, *A. monilatum*, *Gymnodinium catenatum*, *Trieres* (= *Odontella*) *chinensis*, *Coscinodiscus wailesii* and *Mediopyxis helysia*. The validity of these examples is investigated using the available molecular data and our observations on the intraspecific morphological variability. These species categorized as non-indigenous can be divided into two groups, and we conclude that monitoring surveys should focus on the group of less common species with important fluctuations of abundance, independent of tentative labels as exotic or indigenous, because they are potentially useful as bio-indicators of environmental changes.

How to categorize a plankton microbe as non-indigenous?

We can only categorize a planktonic microbe as non-indigenous when we are able to establish where the native or natural population

is, and when we are able to demonstrate a geographical discontinuity between the native and the non-indigenous populations. It is clear that the koala is native to the eucalyptus forests in eastern Australia because there are no natural populations in other continents. More difficult is to establish the locality of a native population when the species is widespread. After numerous studies of fossils and genetics, most anthropologists currently agree that *Homo sapiens* is native from Eastern Africa. We can assume that the native population is found in the place where the species first evolved. However, we cannot easily track the long-term evolution of microbes, as rarely is there a fossil record. We have also to consider the change of the configuration of the continents and ocean current patterns since the species evolved. The consideration of the native population of a planktonic microbe as the place of its evolutionary origin is currently technically unviable.

A second option is to consider the location of the native population as the place where the species is most commonly found, with permanent populations and in relatively high abundance. However, this method requires an exhaustive knowledge of spatial and temporal variability of planktonic microbes throughout the world ocean, which is non-existent at present.

A third option is to consider the type locality as the locality of the native population. The designation of the type locality is a protocol in the description of any new species. It is the place where the type specimen of a new species was collected. Unfortunately, this information is missing or imprecise in some earlier descriptions. For example, Schütt did not report in detail the place of collection in the descriptions of new dinoflagellates [5]. In other cases, two or more distant places are reported in the original description. The diatom *Skeletonema costatum* was described simultaneously from the North Sea and Hong Kong, and only after a detailed study it was found that material from the North Sea corresponded to other species [6]. However, it is not always possible to assign a species to a single type locality. The first microscopes were available in northern Europe, and the earlier microscopists (O.F. Müller, Ehrenberg) examined planktonic microbes from the North or Baltic Seas, often during summer sampling. Consequently, the type locality of numerous species is quite possibly the northern border of their distribution ranges. For example, Meunier described the epiphytic dinoflagellate *Coolia monotis* from the coastal North Sea [7]. Currently, *C. monotis* is common in the Atlantic coasts of southern Europe and the Mediterranean Sea, while it is infrequent in the North Sea and never reported further north. It is evident that Meunier described *C. monotis* from individuals at the marginal range of distribution. The North Sea is the type locality, but it is probably not the site of the native or natural populations of the species. Molecular biology provides means of detecting cases of cryptic speciation: this means individuals that are apparently morphologically identical to each other but the DNA sequences showed that they belong to different species. Molecular data show that the sequences identified as *C. monotis* were divided into two clades. The clade that contained the sequences from individuals isolated from the North Sea is considered the 'true' *C. monotis*, and the sequences of the other clade were assigned to a distinct new species [8]. This does not mean that the native population of *C. monotis* is located in the North Sea.

The type locality is the site associated with the type specimen and should not be the locality where the species was first reported. The dinoflagellate species *Akashiwo sanguinea* was formally described

from Japan in 1922, but it was illustrated in Europe in 1883 as *Gymnodinium gracile* or cited as *Spirodinium fissum* in 1917, and later in 1925 described as *Gymnodinium splendens* [9-11]. Because of its formal description from Japan, *Akashiwo sanguinea* has been categorized as non-indigenous in Europe, but it could have been correctly described first in Europe and then it could have been considered as an exotic species in Japan. The dinoflagellate *Prorocentrum mexicanum* (= *P. rhathymum*) was first described from the Mexican Pacific in 1942, and categorized as non-indigenous in the Mediterranean Sea [1]. However, in 1933 Schiller ([12], his figure 44a) illustrated the species as *P. maximum* from the Mediterranean Sea [13]. The type locality provides information on where a species type specimen was found, but it does not always reflect the natural or native range of a species.

Cosmopolitan or Endemic Distribution of Planktonic Microbes?

The number of non-indigenous planktonic microbes will increase when an endemic distribution predominates, and vice versa. Microbial biogeography is regarded from two points of view: a) the -cosmopolitan or ubiquity hypothesis- assumes a low diversity and cosmopolitan distribution of microbes the -endemism hypothesis- that assumes a high diversity and geographically restricted gene flow with a high number of endemic species [14,15].

The cosmopolitan hypothesis assumes that marine microbes are characterized by large population sizes and, hence, high dispersal probability, which prevents isolation and allopatric speciation. This hypothesis was originally formulated as 'Everything is everywhere, but the environment selects' [16]. 'Everything is everywhere' alludes to the remarkable dispersal potential of microorganisms, whereas 'the environment selects' implies that only specifically adapted organisms will proliferate in a particular environment. We can also connect this view with the 'plankton paradox' briefly: why so many species of plankton? [17]. One of the hypotheses for high diversity in the plankton is that the water column is a constantly changing environment. This mitigates competitive exclusion and favours the high species diversity, as each species will find a set of environmental and ecological conditions to proliferate. Finally, this facilitates geographical dispersal and the cosmopolitan distribution of planktonic species.

On the other hand, the endemism hypothesis is mainly supported by taxonomists of naked ciliates based on the supposed high number of undescribed species, and the high rate of new species descriptions [15]. The identification at the species level of naked ciliates requires a laborious protocol of preservation and staining techniques, and observations at high magnifications. Very few researchers are able to identify naked ciliates at the species level during routine plankton observations with an inverted microscope. For that reason, the records of numerous species remain restricted to the original description, and consequently numerous species are artificially considered as endemic due to the lack of data on their distributions.

Since molecular data are available, especially single-cell PCR for uncultured species, we should be able to solve doubts on biogeography by comparing the DNA sequences of distinct geographical isolates. However, this is not free of problems. There is no unique number of sequence differences indicating that two DNA sequences belong to the same or distinct species. The percentage of divergence also varies according to the molecular marker used (i.e., SSU rRNA versus ITS gene sequences, etc.).

Are there Endemic Planktonic Microbes?

'The environment selects', then it should be species which distributions are restricted to places with especial environmental conditions. This is evident for the extremophiles, as the plankton around the Antarctica. Certainly, the extreme environment conditions have selected cryophilic species (Fig. 1A and B). These endemic Antarctic species are not in the lists of non-indigenous species. Within a context of global warming, we expect more success in the introduction of thermophilic species. Most of these species have a circumtropical distribution as they are known from low latitudes of the Atlantic and Indo-Pacific Oceans. It should be noted that these oceans were connected in recent geological periods. For example, the Panama isthmus that separates the Pacific and Atlantic basins (formed around 2.8 million years ago) is younger than most of the plankton species. The Canals of Suez and Panama have recently interconnected the ocean basins, but these human constructions lacked an environmental impact assessment, and we do not know much about the planktonic microbes at both sides of the Canal of Suez before 1867. The Mediterranean Sea is a semi-enclosed basin where more than one-half of the benthic invertebrates are considered [18]. In contrast, for microbes such as the dinoflagellates there is no a clear example of any endemic species in the planktonic or

benthic forms [19].

An example of endemic tropical species is the bioluminescent dinoflagellate *Pyrodinium bahamense*, known from the Caribbean Sea and the tropical Pacific Ocean (Fig. 1C and D). Temperature is not the only variable that determinates the species biogeography because some basins of the Mediterranean Sea reach tropical temperatures. It would be a touristic attraction to have a Phosphorescent Bay in the Mediterranean Sea, but *Pyrodinium bahamense* lives preferentially near mangrove areas, and that ecosystem is missing in Europe (as well as the chemical compounds derived from the mangrove leaf decomposition). Another endemic species is the dinoflagellate *Dinophysis miles* that is only known from the Indo-Pacific tropical region. Its cell shape and the formation of the chain is highly distinctive (Fig. 1E). Notably both *Dinophysis miles* and *Pyrodinium bahamense* are cyst-producing species, and they are common in the Strait of Malacca. Thousands of cargo ship exchanges the ballast waters near Singapore. Records of the distinctive *Pyrodinium bahamense* or *Dinophysis miles* outside of their natural ranges could represent examples of non-indigenous species, but the lists of non-indigenous species up to date do not include such clear examples.

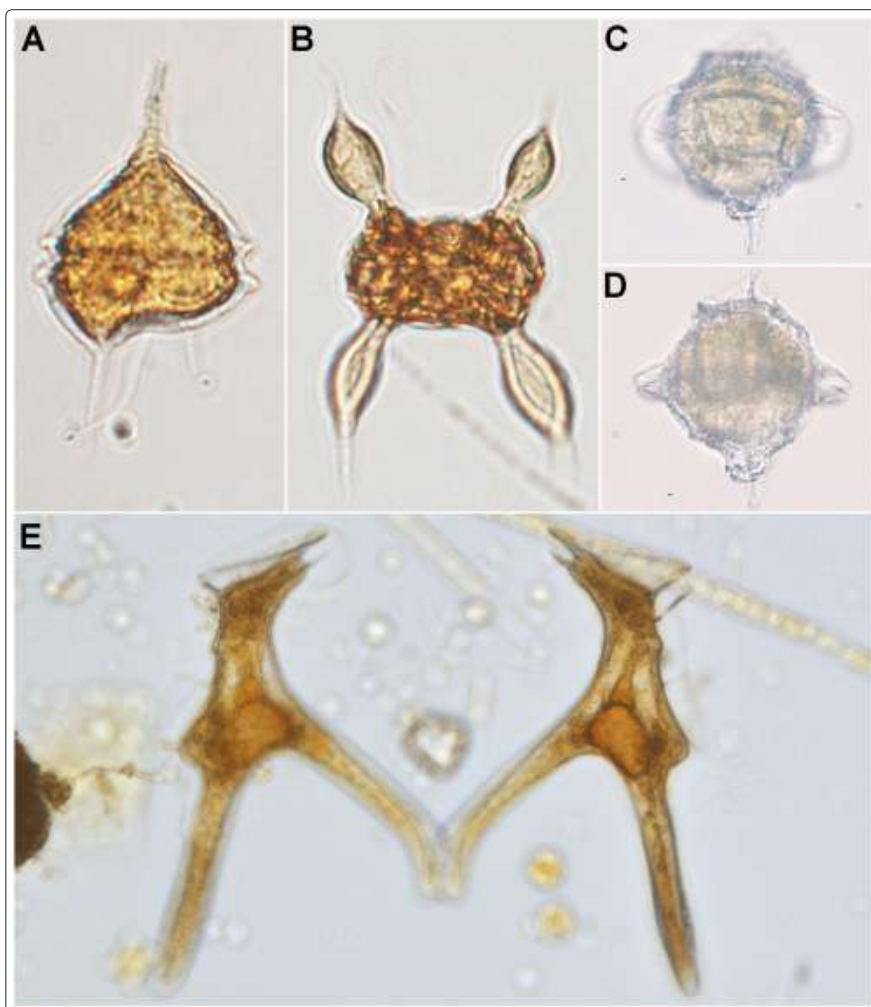


Figure 1: Examples of endemic Antarctic (A-B) and tropical (C-E) phytoplankton. (A) *Protoperidinium defectum* from the Weddell Sea. (B) *Chaetoceros bulbosus* from the Weddell Sea. (C-D) *Pyrodinium bahamense* from the Caribbean Sea. (E-F) *Dinophysis miles* from the South China Sea.

Cases from the Past: Harmful Species

The threat of potentially toxic species such as the dinoflagellate *Alexandrium* is one of the main reasons for the establishment of phytoplankton monitoring programs. The interest increases when the toxic species is also non-indigenous. However, there was a strong bias in the geographical origin of the DNA sequences at the beginning of the use of molecular tools for phytoplankton identification. Most of the DNA sequences were from isolates collected in the coasts of North America, Europe and Japan. Consequently, in the early 2000's, a sequence from a European isolate had two alternatives: to be closer to the sequences available either from North America or Japan. It was clear that it was incorrect to consider that the European isolate was introduced from North America or Japan based exclusively on the similarity of the DNA sequences as the geographical coverage was still too poor. However, this was not consistently an obstacle and Lilly et al. [20] published an article entitled "Paralytic shellfish poisoning toxins in France linked to a human-introduced strain of *Alexandrium catenella* from the western Pacific: evidence from DNA and toxic analysis". The abstract reports: "DNA sequences...

demonstrate that the Thau Lagoon strains...are closely related to populations *Alexandrium catenella*, specifically the Japanese rybotype... introduced via the ballast water of a ship docked at Sète, France". Immediately, the exotic *A. catenella* expanded from France into Spain and Italy as reported in the article entitled "*Alexandrium catenella* (Dinophyceae), a toxic ribotype expanding in the NW Mediterranean Sea" [21]. Lilly et al. were based on the similarity between the strains ATTL01-2 (accession numbers AJ608263-4) from the Thau lagoon, France, and the strain OF101 (accession number U44931) from Ofunato Bay, Japan [20]. If we build a phylogenetic tree, the strains ATTL01-2 are only related to other sequences from the Mediterranean Sea (AJ298900, AJ491289) and distantly related to the Japanese strain (Fig. 2). The invasive *Alexandrium catenella* was most probably never introduced by ballast waters from Asia, and it belongs to a distinct genetic population only known from Mediterranean Sea. To the best of our knowledge, there are no reliable records of planktonic microbes having been introduced through ballast water release [20-22].

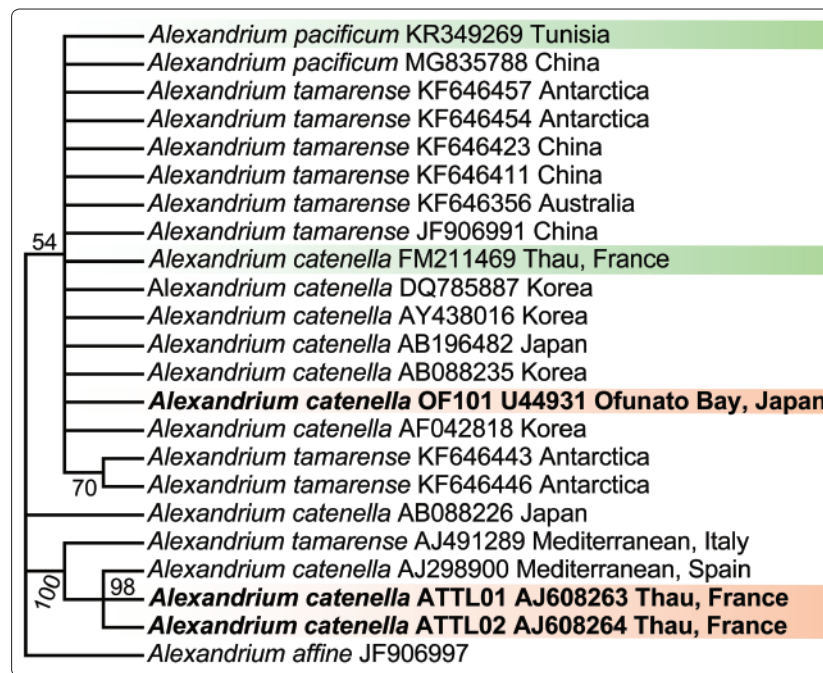


Figure 2: Maximum-likelihood phylogenetic tree of the D1-D2 domains of the LSU rRNA gene sequences of *Alexandrium catenella* with *A. affine* as outgroup. Sequences retrieved from GenBank. Contrary to Lilly et al. [20], the sequences of the strains ATTL01, ATTL02 from Thau lagoon, France, and the strain OF101 from Ofunato Bay, Japan, are not closely related.

Alexandrium monilatum is a species only known from the Caribbean Sea and the tropical eastern Pacific, favoured by the special conditions near mangroves. The waters of the Black Sea are not tropical, and there are no mangrove forests. The supposed blooms of *Alexandrium monilatum* in the Bulgarian coasts would be then a misidentification for other species. According to Elbrächter et al., the first description of a bloom of *Alexandrium* corresponds to *Peridinium splendormaris* observed by Ehrenberg in the Gulf of Naples in August 1858 [23]. These authors also claimed that *Peridinium splendormaris* corresponds with *Alexandrium balechii* that was described from the mangroves of Florida [24,25]. Moreover, as molecular data are available for nearly all the species of *Alexandrium*, but anomalously

missing for *A. balechii* and one can wonder whether this species would be present in the Mediterranean Sea. This is an example of how far we are from documenting the biogeography of planktonic species because even the species responsible of the first documented bloom of *Alexandrium* remains relatively under investigated.

In the late 1970's, human deaths occurred after consuming mussels from the Galician Rias (NW Spain). This prompted establishment of the first phytoplankton monitoring programs, and the causative agent was identified as the dinoflagellate *Gymnodinium catenatum*, first reported in 1976. Before that date, phytoplankton studies in the region were too few to know if *G. catenatum* was a newcomer or

long-time resident. However, hypotheses on the exotic provenance were proposed (i.e., introduction by the Galician fishing fleet which during the 1970's operated in Argentinian waters) [26]. Later, cyst data revealed the presence for, at least, one century ago [27]. Finally, Ribero et al. concluded 'the available evidence points towards natural range expansion, possibly from NW Africa' [28]. The morphology of *Gymnodinium catenatum* is distinctive because no other free-living unarmoured dinoflagellate is able to form colonies up to 64 cells. A non-indigenous and toxic species quickly became a fashionable topic, and made it easier to start looking for it. Although beyond the Iberian Peninsula, no one observed chains of more than four cells, *G. catenatum* was reported in Italy, and as an invader in the North and Baltic Seas [29]. The likely truth would be that *G. catenatum* would have never been there, and that these records would be due to a series of misidentifications. Blooms of *G. catenatum* were a recurrent problem in the 1980-1990's, but at present day only residual populations remain. The species may have important inter-decadal fluctuations in abundance. Rather than an exotic origin, the few previous studies likely simply would have not detected its presence when cell abundance was low. Therefore, research should be focused on the environmental factors that determine fluctuations in the abundance.

The case of *Gymnodinium catenatum* was useful in advancing the taxonomy of the unarmoured dinoflagellates. The misidentifications corresponded to taxa further described as *Gymnodinium impudicum*, *G. nolleri* or *G. microreticulatum*. However, an intensification of the taxonomical studies may result on an over-splitting with questionable new species as occurred with the diatom *Pseudo-nitzschia* [30]. Dinoflagellate research monopolized the monitoring of harmful phytoplankton, while diatom researchers rarely participated in the programs. This fact changed in 1987, with the death of three elderly

people following the consumption of mussels. The intoxication was attributed to the domoic acid in the shellfish produced by species of *Pseudo-nitzschia*. Since then, after 30 years, no deaths have been confirmed due to Amnesic Shellfish Poisoning, the syndrome caused by the domoic acid. Before 1990, the species of *Pseudo-nitzschia* did not receive much attention, and the records were usually pooled as *P. seriata* or *P. delicatissima*. The relatively recent interest in *Pseudo-nitzschia* has resulted in the proposal of 40 new species, with some species indistinguishable from others even using electron microscopy. Each detailed study in a region unavoidably revealed species of *Pseudo-nitzschia* that were first described before in other regions. These "new records" likely inflated the lists of non-indigenous species.

Classical Cases: *Trieres regia* (= *T. chinensis*)

Ship transport as a potential pathway for the introduction of exotic phytoplankton is reported in the literature since the earlier studies [31]. The case of *Trieres chinensis* (= *Biddulphia chinensis*, *Denticella chinensis*, *Odontella sinensis*) is cited as the first example of introduction of a plankton species by ballast waters since 1908 [32]. In 1858, Schultze described *Trieres regia* as *Denticella regia* from the North Sea [33]. In 1866, Greville described *Trieres chinensis* as *Biddulphia chinensis* from samples collected in Hong Kong, but he did not compare his new species with *T. regia* [34]. The main difference between *T. chinensis* and *T. regia* is the relative position of elevation with the ocellus (like a horn), and the labiate processes with long external tube (like a spine) in each valve pole. In *T. chinensis*, the long external tube of the labiate process is close to a slender elevation (Fig. 3A), whereas in *T. regia* the external tube is less prominent and more distant to a less slender elevation (Fig. 3B and C). However, we can find individuals with a valve belonging to *T. regia*, and the other valve to *T. chinensis* (Fig. 3D and E).

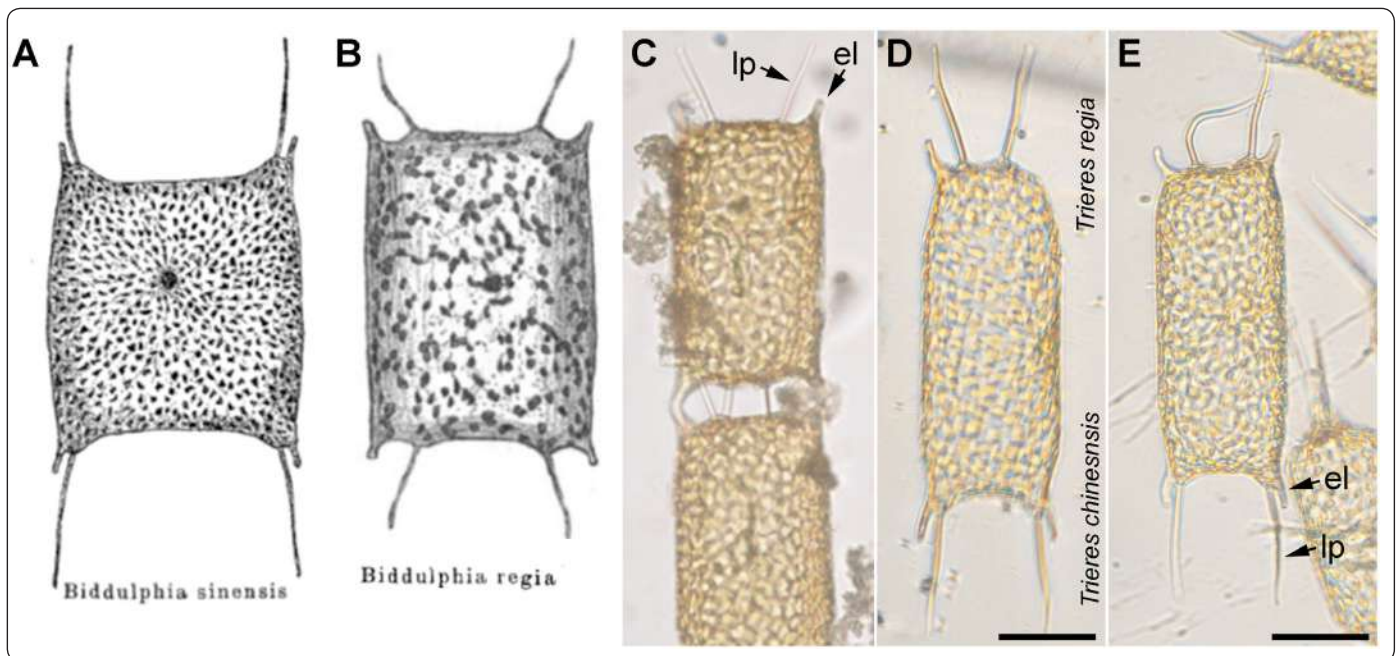


Figure 3: The diatom *Trieres regia* and its synonym *T. chinensis*. (A and B) Line drawings of *T. chinensis* and *T. regia*, respectively, reproduced from Ostenfeld [32]. (C-E) Light micrographs of *T. regia* from the English Channel. (D and E) Note the variability in the distance between the external tube of the labiate process (spine) and the elevation (horn) with an ocellus at each valve pole. Note that one valve correspond to *T. chinensis* and the other valve to *T. regia*. Abbreviations: el = elevation, lp = labiate process. Scale bar = 100 μ m.

Molecular data should resolve these taxonomic questions. The SSU rRNA gene sequences of *T. regia* (accession number KC309502) and *T. chinensis* (HQ912564) available in GenBank are identical (100%), and in a phylogenetic tree, the available sequences of *T. regia* and *T. chinensis* clusters together as they correspond to a single species (Fig. 4) [35]. The exotic provenance of *T. chinensis* in the Atlantic Ocean has then to be discarded, as it is a junior synonym of *T. regia* that was first described from the North Sea.

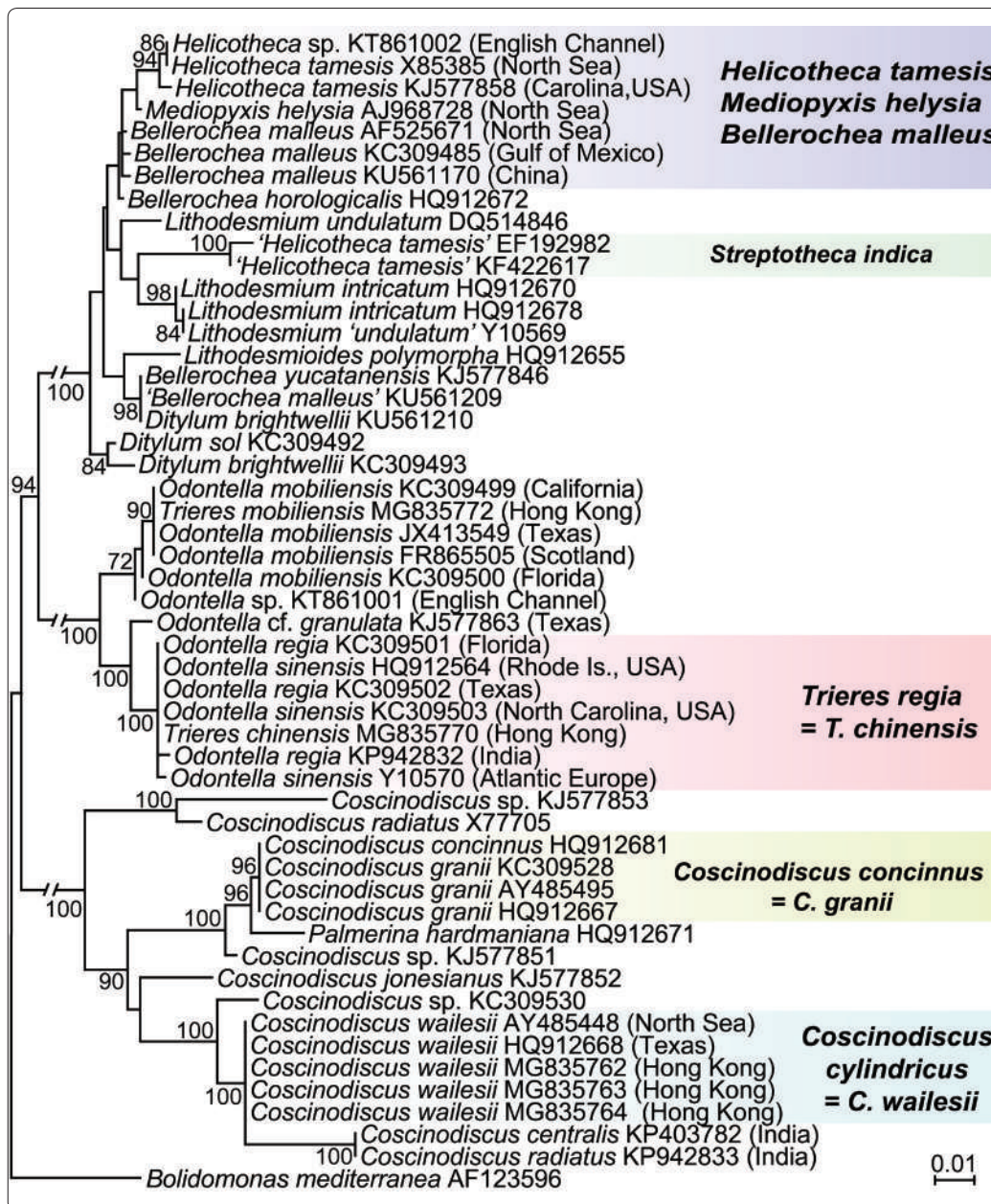


Figure 4: Maximum Likelihood tree of the small subunit rDNA (SSU rDNA) gene sequences of diatoms, with especial focus on the clades of *Trieres*, *Coscinodiscus* and *Mediopyxis*, and *Bolidomonas mediterranea* as outgroup. Sequences retrieved from GenBank. The geographical origin is placed between parentheses.

Ostenfeld began to observe *Trieres chinensis* from August 1903 in the Norwegian North Sea and attributed the presence to a recent introduction from the Indo-Pacific Oceans [32]. Phytoplankton monitoring surveys before 1903 were scarce, and consequently it was premature to categorize any species as alien because of the scarce temporal and spatial sampling coverage at that time. Why has the abundance of the morphotype known as *T. chinensis* increased in the North Sea since 1903? *Trieres chinensis* is the dominant morphotype when the environmental conditions are mild as in summer. Ostenfeld did not observe *Trieres chinensis* from 1899 to 1902 probably because there was a cold climate period in the European Atlantic as revealed by the negative or very low values of the North Atlantic Oscillation (NAO) winter (December to March) index (Fig. 5) [36]. By 1903, the winter NAO index reached the highest positive value since the first available data in 1864 to the 1990's. A climatic shift occurred in 1903 with the wettest record in some parts of North Sea, and the longest recorded period of positive winter NAO indexes (12 years) (Fig. 5) [37]. These mild weather conditions favoured the

proliferation of thermophilic phytoplankton forms. *Trieres chinensis*, the morphotype of the local species *T. regia* began to predominate during the warm period after 1903. Thus, the observations of *T. chinensis* by Ostenfeld were a bio-indicator of a warm climatic episode [32]. In the late XIX century, the colder conditions favoured the form of *T. regia*. At present, *T. chinensis* is the most common morphotype in the European Atlantic [32, 36, and 37].

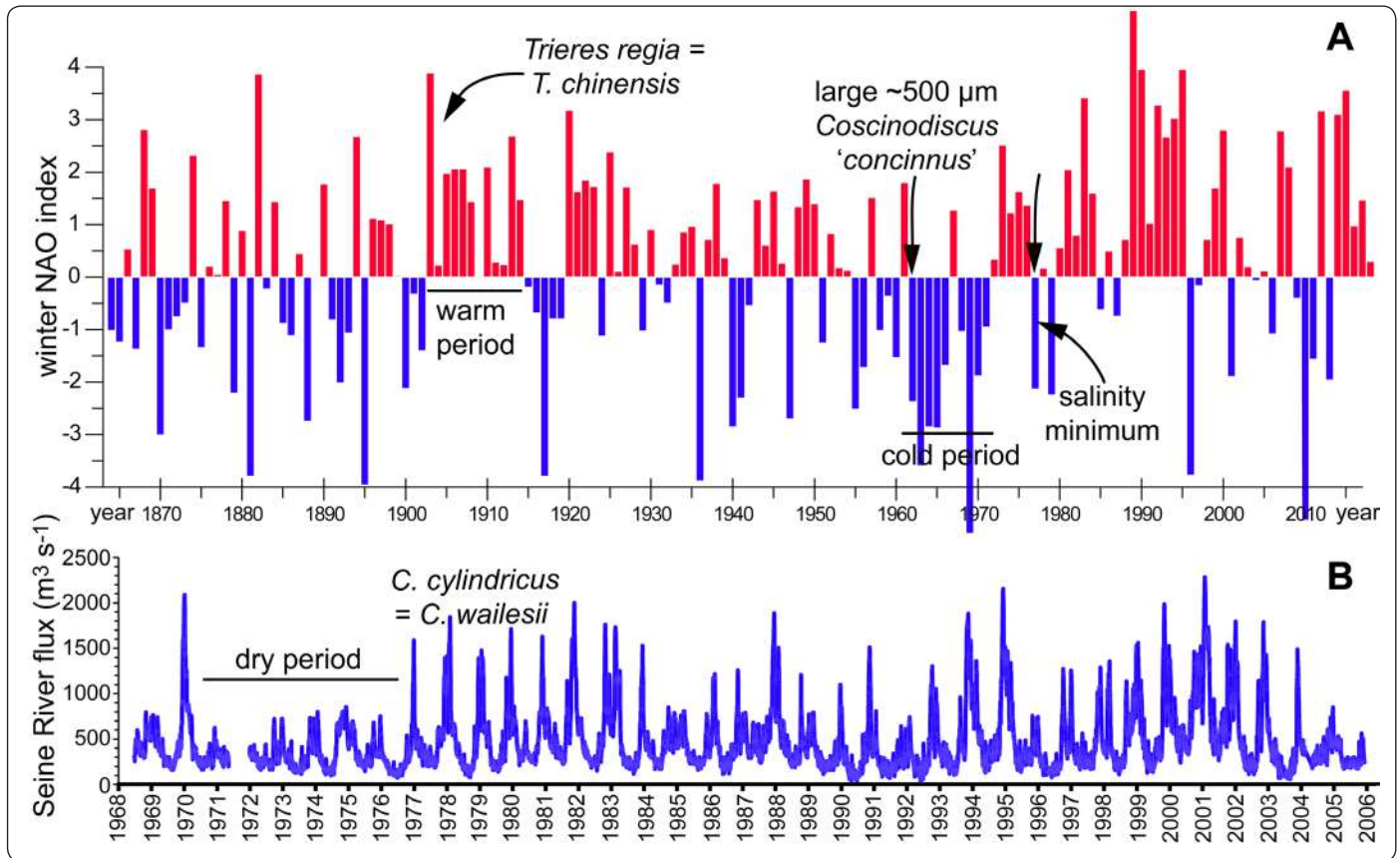


Figure 5: (A) Year to year variation of the North Atlantic Oscillation (NAO) winter index (December-March). Data source: https://climatedataguide.ucar.edu/sites/default/files/nao_station_djfm.txt. (B) Temporal evolution of freshwater discharges of the Seine River at Poses Dam. Data source: Station Hydrometric Station of Poses of DIREN, Île de France

Classical cases: *Coscinodiscus cylindricus* (= *C. walesii*)

Another good example is *Coscinodiscus walesii* reported as a non-indigenous species on both sides of the North Atlantic, and in the South Atlantic Ocean. About 400-500 species of *Coscinodiscus* are currently accepted and the synonymy of the species can be complicated because the earlier descriptions were not very detailed or illustrations are missing [38]. The species of *Coscinodiscus* are coin- or drum-shaped, and some species have a distinctive shape in girdle view, but they settle in settling chambers preferentially showing the valve view (Fig. 6A). Frustules fall in girdle view when there are many cells in the sample (during blooms), if not we have rotated the cells (Fig. 6B). In girdle view, *C. concinnus* showed a distinctive convex dome-shaped valve or an asymmetric valve (wedge-shaped) in its morphotype *C. granii* (Fig. 6B), while cells with a rectangular outline were first identified as *C. nobilis* and later as *C. walesii* (Fig. 6). During decades in the North Atlantic, the taxonomists discussed about the identity of *C. concinnus* and *C. nobilis*, while European researchers described that taxon in the Pacific Ocean as the new species *C. cylindricus* in 1928 and *C. walesii* in 1931 [39,40]. As the identity of *C. nobilis* is poorly defined due to the deficiencies in the original description, the earlier description of this taxon corresponds, in fact, to *C. cylindricus* (see Appendix 1 as Supplementary Material).

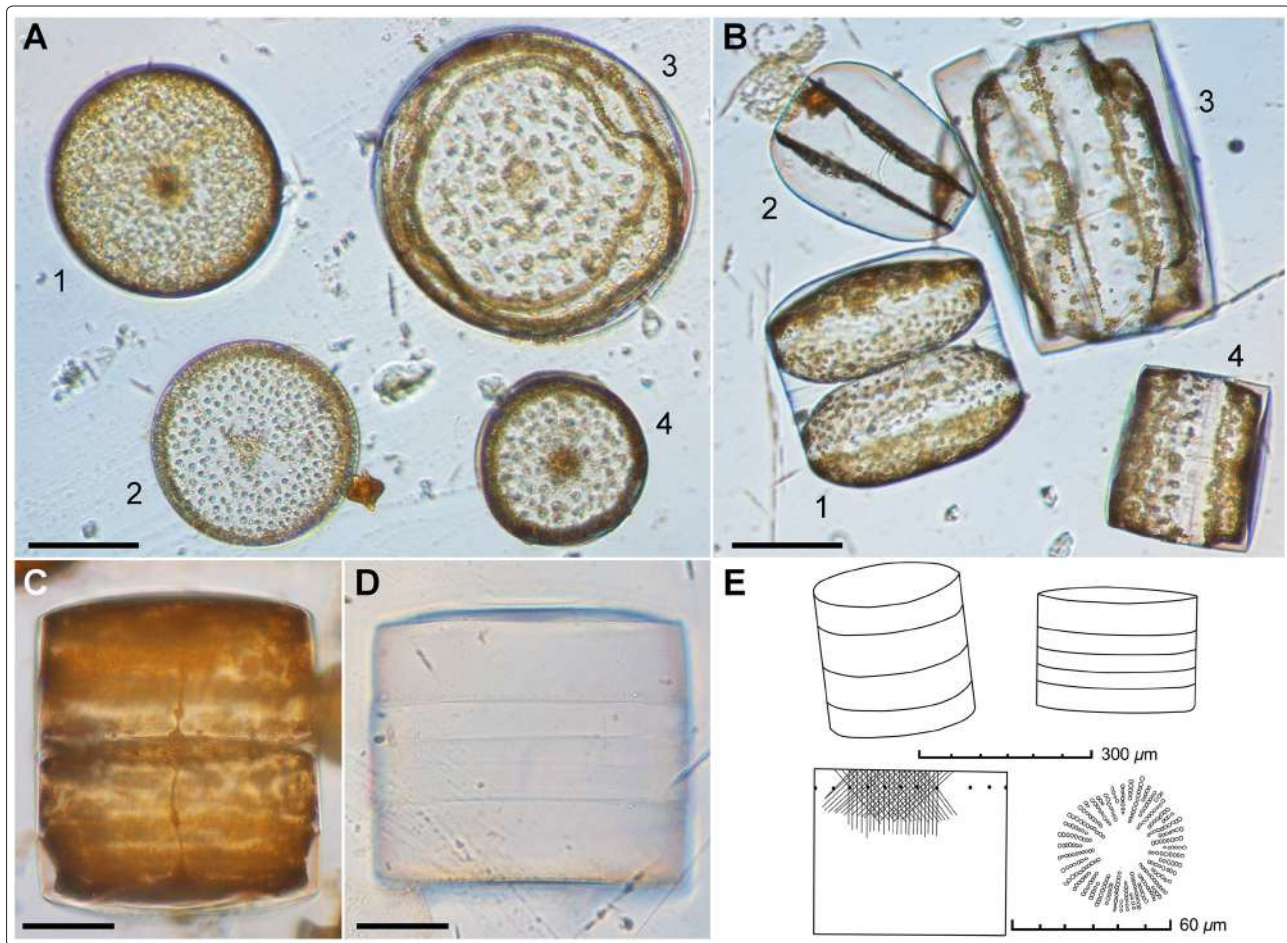


Figure 6: Illustrations of *Coscinodiscus* spp. (A) Individuals in valve view of (1) *Coscinodiscus concinnus*, (2) *C. granii* (= *C. concinnus*) and (3, 4) *C. cylindricus* (= *C. walesii*) from the same sample from the English Channel in 2018. (B) The same individuals in girdle view. Note that *C. cylindricus* (the mantle meets the valve face at right angle) can be smaller or larger than *C. concinnus*. The numbers marked each individual in valve and girdle views. (C and D) *C. cylindricus* from the English Channel in 2018. (E) Original description redrawn from Mangin [39]. Scale bar = 100 µm.

During the vegetative divisions, the centric diatoms experience a decrease of the frustule size until the formation of the auxospore, a cell destined to restore large size individuals in a diatom population. As revealed in culture experiments, cells of *C. cylindricus* with diameters lesser than 300 µm occurred under normal salinity conditions and the frustule diameters higher ≥ 500 µm are associated with low salinities [41]. Likely the salinity stress induces sexual reproduction and the maximum cell size is restored, hence the record of large cells under low salinity conditions. Consequently, under typical marine environmental conditions, *C. cylindricus* is small (250-300 µm) and easily confused with other congeneric species such as *C. concinnus* (Fig. 6A) or *C. gigas*. Blooms of a diatom that reaches a diameter of 500 µm require huge amounts of nutrients, including silica that would only be available under exceptional conditions. Estuaries are shallow environments with high nutrient availability, but high turbidity can reduce light availability and subsequent photosynthetic growth. *Coscinodiscus cylindricus* is able of positive buoyancy when nutrients are available, favouring its proliferation in open waters when the conditions are favourable. [42]. In late 1976 and 1977, climate and oceanographic conditions

were exceptional in the English Channel off Plymouth [43]. A low salinity water mass of Arctic origin arrived in the English Channel in 1977. The years 1973-1976 were dry and with positive values of the winter North Atlantic Oscillation (NAO), but since the summer of 1976 negative values were recorded associated with abundant rainfalls (Fig. 5). This constituted a 'perfect storm' characterized by cold and low salinity waters with important nutrient inputs (i.e., river discharges) [44]. Since the summer of 1976, the residual populations of *C. cylindricus* normally restricted to the estuaries have found favourable conditions to bloom and spread into the open waters of the English Channel. The formation of the auxospores restored the maximal size. Individuals of more than 500 µm dominated then, that were misinterpreted as a newcomer [45]. Similar blooms occurred in the North Sea in 1963-64, after the Big Freeze of 1963. The blooms of a huge diatom of 500 µm in diameter were identified as *C. concinnus* and it was associated with anoxia episodes and invertebrate mortalities, as occurs in the type locality in Japan [47]. *Coscinodiscus cylindricus* is a good biological indicator, but the environmental conditions that favoured the blooms are now infrequent. Currently, the progressive warming,

reduction of eutrophication and river inputs (silica) would certainly decrease the likelihood of massive blooms of *C. cylindricus* in the North Atlantic.

A Recent Case: *Mediopyxis helysia*

European researchers often described new species such as *Coscinodiscus cylindricus* or *C. wailesii* outside Europe [39,40]. To solve the identity of *C. concinnus* or *C. nobilis* in Europe is difficult (are there highly polymorphic taxa or whether these morphotypes belong to independent species), while it is easier to describe a new species from remote regions. In recent years, the molecular techniques have provided tools to solve these doubts. After two centuries of plankton studies in the North Sea, no one expected to find a new large pelagic diatom. However, the molecular analyses of different strains of a supposed high polymorphic species may reveal a cryptic speciation. In 1991, Medlin et al. were pioneers in the use of molecular tool to detect cryptic speciation in marine phytoplankton when they proposed *Skeletonema pseudocostatum* as a distinct species from *S. costatum* [48]. Again, Medlin's molecular data showed that DNA sequences of strains of *Helicotheca tamensis* corresponded to distinct species, and *Mediopyxis helysia* was proposed [49]. Meier et al. entitled their paper as "Dominance of the non-indigenous diatom *Mediopyxis helysia* in Wadden Sea

phytoplankton" where *M. helysia* is presented as a non-indigenous species, and even invasive [50]. The type locality of *M. helysia* is the North Sea, but it was considered as non-indigenous where it was first described. *Mediopyxis helysia* (Fig. 7B-F) was previously mistaken for morphotypes of *Helicotheca tamensis* (Fig. 7A, C-D) and *Bellerocoea malleus* (Fig. 7G and H). These common species in the North Sea and the English Channel are closely related in the molecular phylogenies (Fig. 4). *Mediopyxis helysia* was mistaken for *H. tamensis* or *B. malleus* for over 130 years in the North Atlantic. One should note that misidentifications of diatom strains are unfortunately quite frequent, especially due to the lack of interest to hire taxonomists in many institutions, while most of the current offered jobs require only the profile of molecular biologists. For example, there is another clade with sequences identified as *Helicotheca tamensis* that in fact corresponds to *Streptothecha indica* (Fig. 4). However, DNA sequences identified as *Bellerocoea malleus* are dispersed in five distant clades evidencing that at least four different genera have been misidentified as *B. malleus* [51]. Moreover, the increase of abundance of *Coscinodiscus cylindricus* and the morphotype known as *Trieres chinensis* is associated with climatic events, but the use of *M. helysia* as a climatic bio-indicator is more difficult because in the historical data set it was confused with *H. tamensis* (Fig. 7C and D) and even with *B. malleus* (Fig. 7G and H).

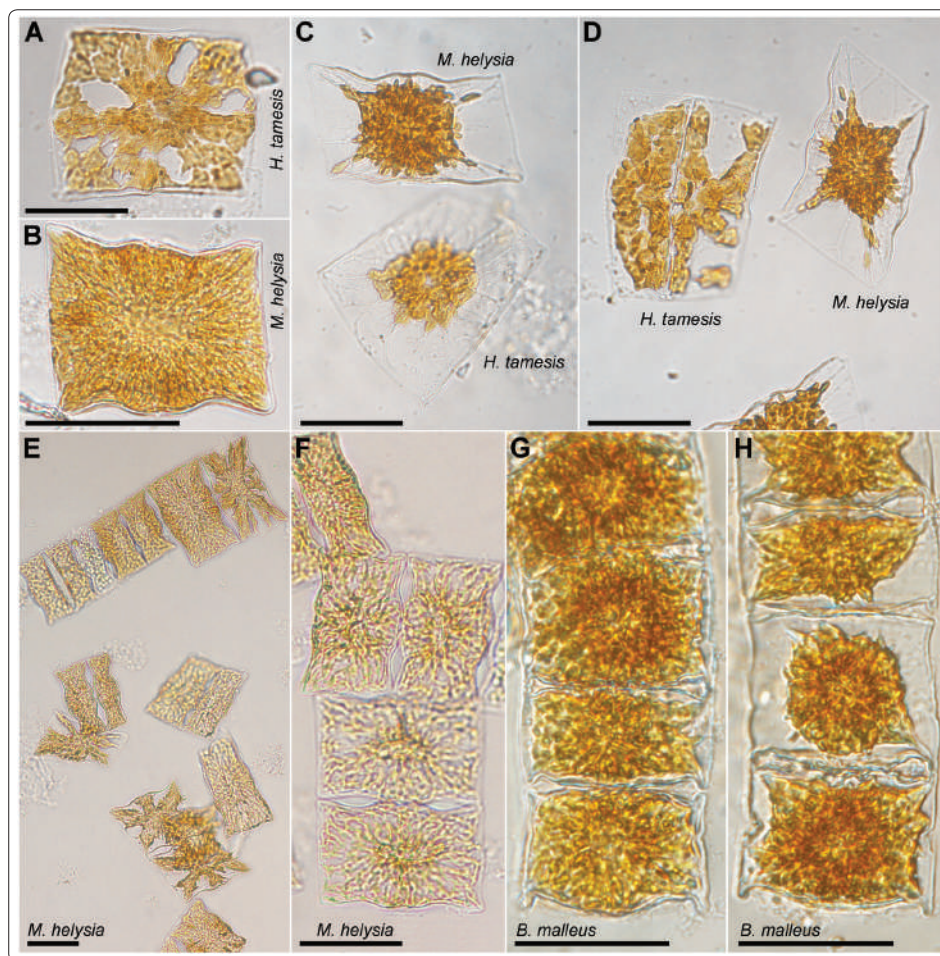


Figure 7: Light micrographs of *Helicotheca tamensis*, *Mediopyxis helysia* and *Bellerocoea malleus* from the English Channel from ECOPEL cruise in summer 2018. (A) *Helicotheca tamensis*. (B) *Mediopyxis helysia*. (C and D) Cells of *H. tamensis* and *M. helysia*. (E and F) Morphological variability of *Mediopyxis helysia*. (G and H) *Bellerocoea malleus*. Scale bar = 50 μ m.

Types of 'Non-Indigenous' Plankton Microbes

A list of non-indigenous species can be artificially inflated with two groups of species. A first group are those that are difficult to identify under routine microscopical observations. A second group contains the species that an experienced observer can identify, but they show important fluctuations in abundance. This implies that the species is undetected for long periods due to its low abundance, until an environmental factor trigger the abundance. Then, the species is mistaken as a newcomer.

In the first group (species of difficult identification), we can find genera which species number have recently increased due to recent detailed taxonomical studies such as *Pseudo-nitzschia* or *Skeletonema*. Other species belong to genera that received less attention such as *Thalassiosira* or *Pleurosigma*. Some species of *Thalassiosira* first described from Japan are listed as non-indigenous in Europe, and other classical example is *Pleurosigma planctonicum*. The identification at the species level of the planktonic species of *Pleurosigma* is difficult, and even we doubt whether the species belong to *Pleurosigma* or *Gyrosigma* during the routine microscopical observations. Among the dinoflagellates, the identification at the species level of delicate unarmoured dinoflagellates of the genera such as *Karenia*, *Karlorinium*, *Takayama* and Raphidophytes require cultures and molecular data for reliable identification. These species are not suitable bio-indicators because it is difficult to track their abundances under routine phytoplankton observations.

More interesting is the second group of species: taxa relatively easy to identify, and characterised by important interannual fluctuations in abundance. These taxa only receive attention when an environmental factor triggers the abundance, and go unnoticed during periods unfavourable to blooms such as *Trieres regia* (= *T. chinensis*), *Coscinodiscus cylindricus* (= *C. wailesii*) or *Gymnodinium catenatum*. They represent potential biological indicators of environmental changes.

Concluding Remarks

Planktonic microbes in tropical and temperate waters tend to have cosmopolitan populations, and there are few examples of endemic species. The vast majority of planktonic microbial species are everywhere, with residual populations that go undetected, receiving attention only when an environmental change triggers the population. These species do not necessarily need human-derived transport pathways for dispersion. However, human activities modify environmental conditions. The exceptional abundance recorded in some periods driven by climatic events should not be confused with the arrival of exotic species.

The categorization of a species as non-indigenous requires knowledge of where the native population is, and demonstration of a geographic discontinuity between the native and the introduced populations. This is difficult because sample coverage and our knowledge of the biogeography of the species have important biases. It is also difficult to determine where the native population is. The type locality is just the place where the specimen of the original description was collected, and it does not represent the native population. The consideration of the place where a species is more common or abundant (as that of the native population) is not always valid. An introduced species, free of its natural predators or parasites, can be more abundant than the native population. The native population is

where the species first evolved, but we do not have the data needed to determinate that place, and also the configuration of the oceans and currents have changed since the species first evolved.

Certainly, there are planktonic microbes that have been transported by humans as occurred with the parasitic dinoflagellate *Amyloodinium ocellatum* that is dispersed by the aquarium and aquaculture activities. The role of human constructions such as the Canal of Suez or Panama on the introduction of planktonic microbes is difficult to evaluate, as we have few data on the plankton microbes at both sides of the Canal before the connection of the ocean basins. Certainly, ballast water is a possible vector for the transport of species, but there is no a clear example of the successful establishment of an exotic microbial species via ballast waters.

More important than focusing on the threat that potential exotic microbial species that would invade our seas, it appears more useful to use these apparently newly resident planktonic microbes as biological indicators of environment changes. A first step would be to characterize in detail the planktonic assemblages, rather than focusing on the few toxic or trendy topic species. The labelling of the planktonic microbes as non-indigenous might be useful to receive funds and to keep running monitoring programs. However, the budget for research being limited, existing resources should be used for establishing detailed biodiversity inventories. Understanding the role of microbes in the oceans has mainly focused on taxa that occur in high abundance. However, marine microbial diversity is largely determined by low-abundance taxa, the so-called 'rare microbial biosphere' [52]. Detailed taxonomical studies in long-term series, coupled to analyses of environmental drivers, are essential to understand the outbursts of these less common plankton species which would be the first witnesses of environmental changes.

Supplementary data

Appendix 1. The case story of *Coscinodiscus cylindricus*

Acknowledgements

F.G. was supported by the convention #2101893310 between the CNRS INSU and the French Ministry of Ecology (MTES-DEB) for the implementation of the Monitoring Program of the European Marine Strategy Framework directive (MSFD) for pelagic habitats and the descriptor 'biodiversity'. Samples were collected during the ECOPEL Manche 2018 spring and summer cruises (CNRS-LOG) onboard R/V 'Antea' (IRD) in the frame of the cited convention and the CPER 'Hauts de France' project MARCO supported by the French state, the 'Hauts de France' French Region and the European Regional Development Funds (ERDF). Thanks are due to captains and crews of research vessels, as well as to the scientific team of both cruises.

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Appendix 1

The case story of *Coscinodiscus cylindricus*

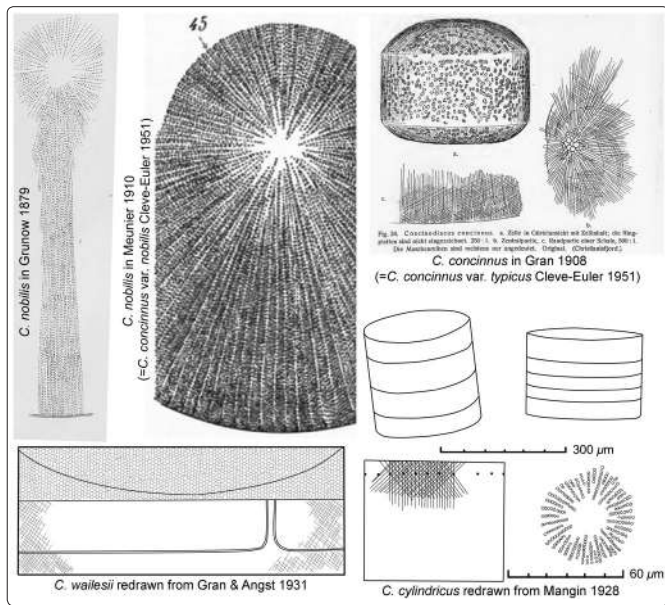
Around 1200 species and infraspecific taxa of *Coscinodiscus* have been described to date, and about 400-500 species are currently accepted (Hasle and Syvertsen, 1997). The synonymy of the species can be complicated because the earlier descriptions were not enough detailed or illustrations are missing. From the English Channel, Smith (1856) described *C. concinnus* without any illustrations. Although the size measurements at that time could be imprecise, Smith remarked that the species was small with a diameter of 64-142

µm. Grunow (1879) described *C. nobilis* from the Java Sea as a huge diatom (540 µm in diameter) with a central hyaline (unperforated) area in the valve. Gough (1905) proposed *C. granii* for cells of *C. concinnus* with an asymmetric valve (wedge-shaped). Gran (1908) illustrated *C. concinnus* as cells of 150-450 µm in diameter with a convex dome-shaped valve and a central areola or rosette. From the Arctic Sea, Meunier (1910) illustrated *C. concinnus* with a central rosette, and *C. nobilis* with a central hyaline area. Meunier (1910, p. 279) commented that according to Van Heurck, *C. nobilis* was a bloom-forming species at Helgoland, North Sea. According to <http://www.marinespecies.org/aphia.php?p=image&pic=16718>, *C. nobilis* sensu Meunier is a synonym of *C. wailesii*. From samples collected in the Inland Sea at Japan, Mangin (1928) described *C. cylindricus* with a diameter of about 300 µm, a central hyaline area in the valve and a rectangular outline in girdle view (the mantle meets the valve face at right angle). From the British Columbia, Gran and Angst (1931) described *C. wailesii* with the same characteristics of *C. cylindricus*. Contrary to Gran (1905, 1908), Cupp (1943) illustrated *C. concinnus* with a hyaline central area. *Coscinodiscus concinnus* and *C. wailesii* were 160-200 µm and 230-350 µm in diameter, respectively (Cupp, 1943). Cupp's monograph of Pacific diatoms was widely distributed, and the observers working in the Pacific Ocean knew the description of *C. wailesii*, whereas the description of *C. cylindricus* was forgotten. In the Inland Sea at Japan, which is the type locality of *C. cylindricus*, the blooms of this huge diatom are commonly identified as *C. wailesii* (Manabe and Ishio, 1991).

In Europe, the descriptions of the exotic species, *C. cylindricus*, and *C. wailesii* did not receive much attention, and the researchers concentrated on the delimitation of *C. concinnus* and *C. nobilis*. Cleve-Euler (1951) merged both species as two varieties. She proposed *C. concinnus* var. 'typicus' for the small forms with a convex valve as illustrated by Gran (1908), and *C. concinnus* var. *nobilis* for the large cells with a flat valve and hyaline central area as illustrated by Meunier (1910). Holmes and Reimann (1966) established a culture of *C. concinnus* and they observed the formation of small cells with asymmetric valves that correspond to the morphology of *C. granii*. Boalch (1971) concluded that the original description of *C. concinnus* included cells of *C. granii*. SSU rRNA gene sequences of *C. concinnus* and *C. granii* available in GenBank (HQ91266, HQ912681) are identical (100%), and molecular phylogeny supports the co-specificity (Fig. 4). Smith (1856) remarked that *C. concinnus* was a small species, but Boalch (1971) reported that *C. concinnus* ranged from 100-400 µm near Plymouth. Also, in Plymouth, Brook reported that *C. concinnus* showed a size range of 100-300 µm in diameter and a dome-shaped valve (Brook, 1975a), whereas *C. granii* showed with a size range of 86-205 µm and an asymmetric valve (Brook, 1975b). On the other side of the English Channel, at Brittany, Paulmier (1971) recorded *C. concinnus* and *C. nobilis*. In the North Sea, blooms of *C. concinnus* cells with a diameter of 465-500 µm have been reported (Hendey, 1964; Peelen, 1965). From the open tropical Indian Ocean, Simonsen (1974) described two closely related species. *Coscinodiscus concinniformis* showed convex valves with hyaline central area and a diameter 150-500 µm. He considered that this species corresponded to the *C. nobilis* illustrated by Meunier (1910) in the Arctic Sea. Simonsen (1974) also described *C. concinnoides* with a similar appearance and a diameter of 300-450 µm.

In 1977, a bloom of a huge diatom caused problems for the fishing activities off Plymouth. Boalch and Harbour (1977) considered that the diameter of that diatom was greater than that previously recorded

for *C. concinnus* in the English Channel. They did not provide size data. Earlier, Boalch (1971) had reported that *C. concinnus* was up to 400 µm in diameter. Therefore, we can assume that the diatom that caused this massive bloom in 1977 was greater than 400 µm in diameter. Boalch and Harbour (1977) did a literature search and identified it as *C. nobilis*. They considered *C. nobilis* as a newcomer, suggesting a recent introduction from Indo-Pacific waters and citing the example of *Trieres chinensis*. However, *C. nobilis* is not necessarily exotic. Meunier (1910, p. 279) reported *C. nobilis* in the Arctic Sea, and commented that the species was responsible for blooms in the North Sea, and Paulmier (1971) recorded it in Brittany. On the American side of the North Atlantic, the same huge diatom has been identified as *C. walesii* (Mulford, 1962; Patten et al., 1963; Marshall, 1971) or misidentified as *C. nobilis* (Griffith, 1961).



Based on Simonsen, Rincé and Paulmier (1986) reported that the species corresponded to *C. walesii*. That diatom was described from the Canadian Pacific (Gran and Angst, 1931), and before as *C. cylindricus* from Japan (Mangin, 1928). Because of diseases of local oysters, oysters from Canada and Japan have been imported since late 1960's to France to replenish diseased farm stocks (Mineur et al., 2014). Rincé and Paulmier (1986) hypothesized that the species came into French coastal waters because of the importation of Pacific oysters. Thus, the species came as *C. walesii* from Canada or as *C. cylindricus* from Japan. However, the micrographs in Paulmier (1997) suggested that *C. walesii* was present before the 1970's. He published micrographs of the diatoms that he observed in Brittany between 1961 and 1972. In the figure 1 of the plate 17, Paulmier illustrated a cell identified as *C. concinnus* from Brittany. In the same plate, he included micrographs of cells of *C. walesii* collected from other French Atlantic areas collected after 1980. The micrographs were taken at the same magnification (×190). Curiously, for Paulmier (1997) *C. concinnus* had a flat valve and it was slightly larger than *C. walesii*. In the original description in the English Channel, Smith (1858) remarked that *C. concinnus* was small (64-142 µm in diameter), but during decades the European researchers have insisted to use the name *C. concinnus* for cells reaching 500 µm, or to identify them as *C. nobilis*. For example, Peelen (1965) reported a massive bloom of cells of 500 µm in diameter identified as *C. concinnus* in the Dutch North Sea. The decay of the bloom was associated with

anoxia and invertebrate mortality (Peelen, 1965). This is the same phenomenon recurrently reported in the Inland Sea of Japan, the type locality of *C. cylindricus* (Manabe and Ishio, 1991). Since the 1980's, there are no more records of *C. nobilis* or blooms of large cells of *C. concinnus* in the North Atlantic. *Coscinodiscus nobilis* has disappeared from the literature, whereas *C. walesii* is widely cited.

After the publication in *Nature* by Boalch and Harbour (1977), a diatom with harmful effects with an exotic origin became a current topic. This diatom can produce anoxia after the massive blooms and its copious amounts of mucilage can clog fishing nets (Boalch, 1987). The huge size of this diatom is an advantage for collection and observation, and the surveys of the Continuous Plankton Recorder with a net of 280 µm pore-size were able to retain that diatom with efficiency, providing data on its abundance and distribution (Robinson et al., 1980; Edwards et al., 2001). Further studies reported that *C. walesii* could modify the pelagic food webs because that species is too big or unpalatable for the herbivorous copepods (Roy et al., 1989), and supersedes native phytoplankton (Rick and Dürselen, 1995; Dürselen and Rick, 1999). Diatoms experts, such as Hasle (1990), already expressed the doubts on the recent introductions of *C. walesii* and *Thalassiosira punctigera* because these species have cosmopolitan distributions and they were already known from the North Atlantic and Arctic Seas. Wiltshire and Dürselen (2004) published an article entitled "Revision and quality analyses of the Helgoland Reede long-term phytoplankton data archive". In their figure 1, the time chart showed records of *C. walesii* before 1977, the date of its 'official' arrival into Europe, but they reported: "data are not possible -species only appeared in the German Bight since the end of the 70s -later changes were made in the electronic data". Wiltshire and Dürselen (2004, p. 254) reported for *C. walesii*: "The species only appeared for the first time at the end of the 1970s in European waters. Prior data are definitely wrong in the database! Later changes must have been made. Supposition: size classes with diameter 270 µm and/or 320 µm were changed to *C. walesii*. However, this coincided with supposed changes from *C. concinnus*".

Notwithstanding these assumptions, there is no reason to consider that *C. cylindricus* (= *C. walesii*) was introduced in the North Atlantic in the 1970's. In the past, that species was misidentified as *C. nobilis* or as large cells of *C. concinnus*. As *Coscinodiscus* cells settle in valve view, during the routine phytoplankton analysis, few cells are observed in girdle view, which shows the distinctive rectangular outline (the mantle meets the valve face at right angle, Fig. 6). *Coscinodiscus cylindricus* resembles *C. nobilis*, which was described with an almost flat valve face (Grunow, 1879). The presence of a hyaline central area in *C. cylindricus* is an unstable diagnostic character because the formation of a central rosette has been also reported (Schmid, 1990; Fernandes et al., 2001).

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